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1 Postprandial glycemic response in different ethnic groups in East London and its association with
2 vitamin D status: study protocol for an acute randomized crossover trial

3 Abstract

4 Background

5 In the UK, Black African-Caribbeans (ACs) and South Asians (SAs) have 3–6 times greater risks
6 of developing type-2 diabetes mellitus (T2DM) and significantly higher prevalence of vitamin D
7 (vitD) deficiency than White Caucasians. East London is among the areas with the highest
8 prevalence of T2DM and the highest proportion of ethnic minority groups. This ethnic health
9 inequality is ascribed to socioeconomic standing, dietary habits, culture, and attitudes, while
10 biological diversity has rarely been investigated.

11 Aim

12 The study aims to investigate the difference in the postprandial glycemic response (PGR), an
13 independent risk factor of T2DM, between ethnic groups (White Caucasians, SAs, and ACs) in
14 East London and its association with vitD status.

15 Methods

16 This acute randomized crossover trial will recruit healthy adults (n=106) in East London between
17 November 2023 and March 2025. Two test drinks are consumed by participants (a glucose drink
18 containing 75 g glucose and pure orange juice) on different occasions. PGRs are monitored before
19 and after drinking every 30 minutes for up to 2 hours via finger prick. A fasting blood sample
20 obtained via phlebotomy will be used for plasma 25(OH)D and relevant tests. A
21 knowledge/perception questionnaire about vitD and a 4-day food diary (analyzing vitD dietary
22 intake) will also be collected. Data will be analyzed using a multiple linear regression model
23 adjusted by confounding factors (age, gender, body mass index, and body fat percentage).

24 Summary

25 The study results will be disseminated through journals and conferences, and target stakeholders.

26 Keywords: postprandial glycemia, type 2 diabetes, oral glucose tolerance test, 25(OH)D, ethnic
27 minority, body fat percentage, body mass index

28 Introduction

29 Health patterns differ significantly between ethnic minority groups and the White population. In
30 the UK, the risk of developing diabetes is 3-6 times greater in South Asians (SAs) and up to three
31 times greater in Black African-Caribbeans (ACs) than in White Caucasians, and people in these
32 groups develop this condition at a younger age (Meeks et al., 2016). East London is among the
33 areas with the highest proportion of ethnic minority groups (Gov.uk, 2022a) and the highest
34 prevalence of type 2 diabetes mellitus (T2DM) (Diabetes UK, 2021). Although multiple factors,
35 including socioeconomic standing, diet, culture and attitudes, language barriers, genetics, and
36 lifestyles, have been identified (Hanif & Susarla, 2018), research into biological diversity is
37 scarce. Recent research revealed that the postprandial glucose peak in SAs is two- to three-fold
38 greater than in White Caucasians after identical carbohydrate loads are reached (Tan et al., 2017).
39 Although obesity is believed to account for 90 % of the risk of developing T2DM due to obesity
40 causing insulin resistance (Grant et al., 2021) and some ethnic minority groups, e.g., Black people,
41 have a higher prevalence of overweight and obesity than White British people do (73.6 % vs. 63.3
42 %) (Gov.uk, 2020), other biological mechanisms, including vitamin D (vitD) deficiency, are
43 poorly understood.

44 VitD deficiency in ethnic minorities in the UK is well known and is described as an unrecognized
45 epidemic (Darling, 2020). In the UK, 50 % of SAs and 33 % of Black ACs demonstrate vitD
46 deficiency, whereas 17.5 % of White Caucasians do (Sutherland et al., 2021), which is primarily
47 due to more subcutaneous pigmentation that absorbs ultraviolet B from sunlight and reduces vitD
48 production in the skin and at high latitudes in the UK (Yousef et al., 2023). This situation is worse
49 in East London. In Tower Hamlets, a borough of East London, 80-97 % of the residents are
50 thought to have vitD deficiency, significantly higher than the national population at 50 %
51 (Towerhamlets.gov.uk, 2017). An inverse association of serum 25(OH)D levels with insulin
52 resistance was observed in healthy adults (Yin et al., 2022) and diabetic patients (Abubaker et al.,
53 2022). Recent evidence from systematic reviews and meta-analyses of randomized controlled trials
54 (RCTs) suggests that vitD supplementation may help reduce fasting plasma glucose
55 concentrations, HbA1c levels, and insulin resistance in patients with type 2 diabetes mellitus
56 (T2DM) (Farahmand et al., 2023; Afraie et al., 2024). However, little evidence is available that
57 focuses on ethnic minority groups or residents in East London, indicating that AC and SA
58 communities are underrepresented in the evidence base concerning diabetes and vitD.

59 VitD plays important roles in calcium metabolism and is involved in the modulation of cell

60 growth, neuromuscular and immune function, and the reduction of inflammation due to its
61 receptors being expressed ubiquitously in nearly all human cells, including pancreatic β -cells (Lips
62 et al., 2017). Animal studies have shown that vitD treatment improves insulin production and
63 sensitivity via decreasing inflammatory response and regulating glucose homeostasis (Zhang et al.,
64 2024), or via increasing intracellular calcium in pancreatic β -cells and other metabolic pathways
65 (Szymczak-Pajor et al., 2020). Moreover, 1,25(OH) $_2$ D (the active form of vitD) may modulate β -
66 cell growth and differentiation (Lips et al., 2017). The secondary high parathyroid hormone (PTH)
67 concentration (Malik et al., 2020) and increased inflammatory markers (Zhou et al., 2023)
68 associated with vitD deficiency may also cause glucose intolerance. VitD may have an indirect
69 effect on glycemic control via obesity. Previous evidence (Rafiq & Jeppesen, 2018) revealed a
70 significant inverse association between body mass index (BMI) and serum 25(OH)D, which is
71 thought to involve a complex of mutual influences because vitD receptors are expressed on
72 adipose cells and regulate their functions (Bennour et al., 2022), indicating that vitD deficiency
73 might be one of the causes of obesity, thus indirectly leading to an increased risk of T2DM.

74 The postprandial glycemic response (PGR) has important implications for the development of type 2
75 diabetes mellitus (T2DM) (Shibib et al., 2024). The oral glucose tolerance test (OGTT) is widely
76 used to assess insulin sensitivity and pancreatic β -cell function and to assess an individual's
77 metabolic capacity to handle carbohydrate-containing foods (American Diabetes Association,
78 2013). However, a recently published study indicated that within-subject variations in the PGR
79 pattern may exist between OGTT and food intake, suggesting the necessity of combining OGTT
80 and a meal/drink tolerance test for individualized glycemic management (Liu et al., 2022). There
81 is poor awareness about vitD and its impact on health in the UK. Although the COVID-19
82 pandemic has brought increased public attention to vitD, only around one in six individuals in the
83 UK have been reported to take a daily vitD supplement (Gov.uk, 2022b). However, no such data is
84 currently available for ethnic minority groups or residents of East London. We are also interested
85 in dietary vitD intake between different ethnic groups in East London, which will partly explain
86 the vitD status of the target population.

87 Overall, the evidence on the association of vitD and the risk factors of T2DM in the ethnic
88 minority groups is scant. There is an urgent call for research on ethnic minority populations to
89 address health inequality (The NHS Race and Health Observatory, 2021). This proposal aims to
90 respond to the above call by focusing on ethnic minority communities in East London via investigating
91 the association between vitD status and PGRs among White Caucasian, South Asian (SA), and African
92 Caribbean (AC) adults. The objectives of the study include 1) investigating PGRs to an OGTT, as well

93 as to the consumption of a commonly consumed drink, pure orange juice (OJ); 2) measuring
94 parameters in the blood including 25(OH)D (an indicator of vitD status), and other relevant tests
95 including calcium, PTH, blood lipid profile, inflammatory parameters; 3) assessing the knowledge
96 and perception of vitD; 4) evaluating the dietary vitD intake, in White Caucasian, SA and AC
97 adults.

98 Method

99 This is an acute, randomized, repeated measures crossover trial. Figure 1 shows the study flow
100 chart. The proposal was approved by the Senate Research Ethics Committee at City St George's,
101 University of London (Ref ETH2223-2000). This study was registered with ClinicalTrials.gov
102 (identifier: NCT06241976). The recruitment period of the study is between 1st November 2023
103 and 31st March 2025 in London, UK. The informed consent is obtained from all participants before
104 taking part in the study.

105 Study status: A) participant recruitment will be completed at the end of March 2025; B) data
106 collection will be completed at the end of March 2025; C) results are expected in April and May
107 2025. None of these stages have already been completed.

108 Participants

109 The inclusion criteria are as follows: 18–65 years in general good health and living in East London
110 from White, SA or Black AC origins. The exclusion criteria are as follows: diabetes; digestive system
111 diseases; BMI < 18.5 kg/m²; liver or kidney disease; other chronic diseases; blood clotting disorders;
112 alcohol consumption (>14 units per week); regular smoking (one or more cigarettes per day);
113 pregnancy; maternity; and mixed race. A self-developed health and lifestyle questionnaire (Appendix
114 1) will be used to screen the eligibility of the participants. Participants will provide informed consent
115 online before being screened for eligibility.

116 Recruitment

117 There are a few methods of recruiting participants. We will recruit staff and students who live in
118 East London with gatekeeper permissions from the Dean of the school. Recruitment adverts will be
119 circulated to staff and students at City St George's, University of London. There are 19975 students,
120 among whom 64 % are from the UK, and a large proportion of students are from different London
121 boroughs, including the East London area. Each year, many staff and student projects recruit
122 participants successfully in this way. In addition, we recruit participants from local communities in
123 East London with gatekeeper permission. We will contact local ethnic communities, including the

124 Bangladeshi Community, London Central Mosque, Bangladesh Embassy and Indian & Bangladesh
125 Hindu Community East London, etc. We will also recruit participants via social media, including
126 Meta (formerly Facebook), X (formerly Twitter), Instagram, and LinkedIn. To encourage taking
127 part in the study, participants will receive a shopping voucher worth £20 per visit as an incentive.

128 Study design

129 The participants will consume a glucose drink (75 g glucose in 300 ml water, 281 kcal) used for
130 the OGTT (Eyth et al., 2023) and pure OJ (Tesco 100 % Pure Squeezed Orange Juice Smooth 300
131 ml containing 129 kcal, 30 g sugar, 0.3 g fiber, 1.8 g protein and 90 mg vitamin C) on separate
132 occasions with at least 48-hour interval and at random order. The two drinks were chosen rather
133 than meals because of fewer facilities needed to cater to participants, fewer potential food hygiene
134 issues, and being more acceptable to participants from different ethnic backgrounds. The
135 participants fast for 8–12 h. The blood glucose concentration is measured via a HemoCue Glucose
136 201+ Analyzer (Health-care Equipment & Supplies, Surrey, UK) at 0, 30, 60, 90, and 120 minutes
137 before and after drink consumption by finger prick. Seven milliliters of fasting blood will be
138 collected via phlebotomy on the first visit. If blood collection is unsuccessful on the first visit, it
139 will be attempted on the second visit. The plasma is separated by centrifuging the blood sample at
140 $2000 \times g$ for 10 min and stored at -20°C until analysis of the metabolic parameters (refer to the
141 section on outcome measures).

142 Participants are asked to consume the drinks (either a glucose drink or OJ) within five minutes in
143 the resting area outside the clinic room. The researcher will record the start and end times of
144 consumption. During the 2-h study period, the participants are asked to remain sedentary and
145 refrain from eating or drinking while sitting in the resting area. On the evening before each study
146 visit, participants are encouraged to consume similar meals across both visits, ensure adequate
147 sleep, and avoid alcohol consumption and strenuous physical activity. On the study visit, the
148 researcher will collect information on the participant's dinner from the previous evening, hours of
149 sleep, alcohol intake, and physical activity.

150 The name, email/mobile (for appointment purposes), sex, age, and ethnicity of the participants are
151 also collected. The research assistant (ST) will generate the allocation sequence, enroll participants,
152 collect consent forms, assign participants to interventions and conduct the trial. The study is not blind
153 to the researcher and participants but is blind to the statistician (SI) who will analyze the data.

154 Randomization

155 The order of drink consumption is randomized by using the Excel RAND function. In an Excel

spreadsheet, a list of participant numbers is shown in one column. In the next column, we use the RANDBETWEEN function and choose 0 and 1 as the ranges to randomly generate values of 0 or 1. Participants with a value of 0 will consume a glucose drink first, while participants with a value of 1 will consume pure OJ as their first drink.

Outcome measures

The primary outcome will be the association of plasma 25(OH)D (the most commonly used indicator of vitD status) with the PGRs represented as the incremental areas under the curve (iAUCs) and peak values (PVs) in different ethnic groups. The secondary outcomes include the difference in iAUCs, PVs, and the following variables relating to vitD status between ethnic groups. CRP is reported to have an inverse relationship with 25(OH)D, indicating an anti-inflammatory property of vitD (de Oliveira et al., 2017). The total cholesterol and high-density lipoprotein (HDL) have inverse and positive associations, respectively, with vitD status (Alkhatatbeh et al., 2019). BMI and body fat percentage are used as confounding factors of 25(OH)D (Grønborg et al., 2015) and PGRs to drinks (Dhaheri et al., 2010). PTH and calcium are closely related to vitD metabolism (Goltzman et al., 2018). The plasma 25(OH)D and PTH are measured using an AIA-900 immunoassay analyzer (Tosoh Bioscience, USA); C-reactive protein (CRP), calcium, total cholesterol and HDL are measured using Horiba Pentra 400 Biochemistry Analyzer (Horiba, Japan). The body weight and fat percentage are measured with dual-frequency bioelectrical impedance analysis (BIA) technology by TANITA DC-360 P (Tanita, Amsterdam), and body height by stadiometer. Dietary vitD intake will be analyzed by a four-day estimated food diary analyzed by Nutritics software (Nutritics Ltd., Dublin). Knowledge and perception of vitD will be assessed by an online questionnaire (Appendix 2) collected via Qualtrics survey platform (Qualtrics, Provo, UT).

Data analysis

The sample size was calculated using G*Power software (version 3.1.9.7; Heinrich Heine University, Düsseldorf, Germany), based on F-tests for ANOVA: repeated measures, between-subjects factors. This study aims to achieve an effect size of 0.25 in the PGRs among three ethnic groups, considering that the response is taken from five different time points with 30-minute intervals for each person, and to achieve 80 % power in the study, we will need 32 people in each group (n=96 in total) at the 5 % level of significance. The details of the sample size calculation can be found in Appendix 3. Considering the acute nature of the study, we believe 10 % of the attention is sufficient, therefore, the target number of recruitments is 106 participants. Data normality will be assessed using the Kolmogorov–Smirnov test. Continuous variables with a normal distribution will be presented as means $\pm$ standard deviations (SDs), while non-normally distributed data will be

189 reported as medians and interquartile ranges (IQR). Categorical data are presented as numbers and
190 percentages. The iAUC is calculated using approximated trapezoidal numerical integration (Chlup
191 et al., 2008), and only the incremental area above the fasting level will be included. The association
192 of plasma 25(OH)D concentrations and the iAUCs and PVs respectively, will be analyzed using a
193 multiple linear regression model adjusted by age, gender (using dummy variables), BMI, and body fat
194 percentages, including all participants, and in different ethnic groups. The iAUCs and PVs
195 concentrations between ethnic groups after each drink consumption will be analyzed by one-way
196 ANCOVA adjusted by confounding factors including fasting glucose concentration, age, sex, BMI,
197 and body fat percentage. The correlation of iAUCs and PVs between the two drink consumptions is
198 analyzed by Pearson's correlation to identify the difference in the pattern of PGRs to different drink
199 consumptions. Other continuous variables (e.g., plasma 25(OH)D etc.) between ethnic groups will
200 be analyzed by one-way ANCOVA adjusting confounding factors (age, gender, BMI and body fat
201 percentage). Categorical variables, e.g., gender, the percentage of patients with vitD deficiency etc.,
202 are compared between ethnic groups via Chi-square tests. The statistical significance will be reported
203 with a *P*-value (significance was set at $P \leq 0.05$, two-tailed) and a 95 % confidence interval. The
204 statistical software IBM SPSS 29 will be used to analyze the data. The percentage of missing data is
205 expected to be low based on our previous experience. Therefore, regression imputation is used to
206 address missing data. Both intention-to-treat analysis and per-protocol analysis will be used for the
207 data analysis.

208 Advisory board

209 An advisory board will include senior staff members from the Ethics Committee and the research
210 centers at the school and the principal investigator. The advisory board will meet every three months
211 and make sure the following are checked and in place: safeguard participants in research; protect
212 researchers/investigators (by providing a clear framework to work within); enhance ethical and
213 scientific quality; minimize risk; monitor practice and performance; promote good practice and
214 ensure lessons are learned.

215 Dissemination of the study

216 The findings of the study will be communicated to other researchers, clinical professionals, and
217 policymakers primarily through publications in peer-reviewed journals, seminars, and conferences.
218 A summary leaflet will be produced in plain English and shared with participants, local
219 communities, GP clinics, and hospitals. The leaflet will include actions that can be taken by East
220 London residents in their local food environments. The findings will also be disseminated through

221 university newsletters and the university’s official social media accounts, including Meta, X,
222 Instagram, LinkedIn, and YouTube.

223 Implications of the study

224 The study outcomes will help address the existing research gap regarding the relationship between
225 vitD, PGRs and ethnicities in the UK. The study will raise awareness of the health implications of
226 vitD deficiency, particularly its association with T2DM, and provide rationales to inform
227 educational programmes and food fortification strategies aimed at addressing vitD deficiency in
228 ethnic minority populations in East London and beyond. Though the study is observational in nature,
229 it is anticipated that a major outcome of this study will be evidence to inform a RCT to confirm the
230 causal relationship of vitD status and glycemic control in ethnic minorities in the UK. Currently,
231 research on vitD supplementation and PGR interventions has produced inconclusive results, and
232 research on ethnic minority groups is needed (Christides, 2022).

233 Limitations and future perspectives

234 There are several limitations to this proposal. Firstly, the use of multiple finger-prick tests to
235 measure postprandial blood glucose concentrations at 30-minute intervals may miss the actual
236 peak glucose value. Using 15-minute sampling interval or continuous glucose monitoring (CGM)
237 (Shields et al., 2024) would obtain more precise PGRs. Secondly, the proposal focuses on
238 participants residing in East London; therefore, findings related to knowledge and perceptions of
239 vitD, dietary intake, and the prevalence of deficiency may have limited generalizability to
240 populations outside of this geographic area. In future studies, CGM (Shields et al., 2024) could
241 replace finger-prick testing to assess PGRs following meal or drink consumption. This approach
242 would provide more accurate and comprehensive data while reducing participant burden, provided
243 budget constraints are not a limitation. Additionally, a larger-scale study involving a more
244 representative sample of diverse ethnic groups recruited from multiple London boroughs is
245 warranted to enhance the generalizability of the findings.

246 **Authors’ contributions**

247 HD is the principal investigator who led the study design and the preparation of this manuscript
248 and applied for ethics approval. ST contributed to creating the questionnaire on Qualtrics, and the
249 recruitment advert, CR and SS contributed to developing the study protocol. SI contributed to the
250 sample size calculation and statistical methods. All authors contributed to the preparation of this
251 manuscript and approved the final manuscript. The funder played no role in the preparation of this

252 manuscript.

253 **Data management** (Appendix 4)

254 **Timelines** (Gantt Chart, Figure 2)

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