



**QUEEN'S
UNIVERSITY
BELFAST**

Serum 25-hydroxyvitamin D and insulin resistance in people at high risk of cardiovascular disease: a euglycaemic hyperinsulinaemic clamp study

Wallace, I. R., McKinley, M. C., McEvoy, C. T., Hamill, L. L., Ennis, C. N., McGinty, A., ... Hunter, S. J. (2016). Serum 25-hydroxyvitamin D and insulin resistance in people at high risk of cardiovascular disease: a euglycaemic hyperinsulinaemic clamp study. *Clinical Endocrinology*. DOI: 10.1111/cen.13100

Published in:
Clinical Endocrinology

Document Version:
Peer reviewed version

Queen's University Belfast - Research Portal:
[Link to publication record in Queen's University Belfast Research Portal](#)

Publisher rights

© 2016 John Wiley & Sons Ltd

This is the peer reviewed version of the following article: Wallace, I.R., McKinley, M.C., McEvoy, C.T., Hamill, L.L., Ennis, C.N., McGinty, A., Bell, P.M., Patterson, C.C., Woodside, J.V., Young, I.S. and Hunter, S.J. (2016), Serum 25-hydroxyvitamin D and insulin resistance in people at high risk of cardiovascular disease: a euglycaemic hyperinsulinaemic clamp study. *Clinical Endocrinology*, which has been published in final form at <http://onlinelibrary.wiley.com/doi/10.1111/cen.13100/abstract>. This article may be used for non-commercial purposes in accordance with Wiley Terms and Conditions for Self-Archiving.

General rights

Copyright for the publications made accessible via the Queen's University Belfast Research Portal is retained by the author(s) and / or other copyright owners and it is a condition of accessing these publications that users recognise and abide by the legal requirements associated with these rights.

Take down policy

The Research Portal is Queen's institutional repository that provides access to Queen's research output. Every effort has been made to ensure that content in the Research Portal does not infringe any person's rights, or applicable UK laws. If you discover content in the Research Portal that you believe breaches copyright or violates any law, please contact openaccess@qub.ac.uk.

Serum 25-hydroxyvitamin D and insulin resistance in people at high risk of cardiovascular disease: a euglycaemic hyperinsulinaemic clamp study.

IR Wallace^{1,2}, MC McKinley¹, CT McEvoy¹, LL Hamill¹, CN Ennis¹, A McGinty¹, PM Bell², CC Patterson¹, JV Woodside¹, IS Young¹, SJ Hunter².

¹Centre for Public Health, Queen's University Belfast, Institute of Clinical Science B, Grosvenor Road, Belfast, BT12 6BJ, UK.

²Regional Centre for Endocrinology and Diabetes, Royal Victoria Hospital, Grosvenor Road, Belfast, BT12 6BA, UK.

Short title: Vitamin D and insulin resistance

Keywords: Vitamin D, insulin resistance, euglycemic hyperinsulinemic clamp

Correspondence:

Dr Ian R Wallace,
Regional Centre for Endocrinology and Diabetes,
Royal Victoria Hospital,
Grosvenor Road,
Belfast.
BT12 6BA, UK.

Tel: +44 (0)28 9063 4462

Fax: +44 (0)28 9031 0111

E-mail: iwallace01@qub.ac.uk

Acknowledgements:

We are grateful to Mr Cyril McMaster and Dr Charlotte Neville, Centre for Public Health, Queen's University Belfast for assistance with laboratory analysis.

The authors wish to thank all the participants in the study for their time, interest, co-operation and contribution to this research.

Funding:

This research was funded by the Food Standards Agency, Department of Health, UK.

Conflicting Interests:

The authors have nothing to disclose.

Financial disclosure:

The authors have nothing to disclose.

Word count: 3025

Abstract word count: 210

References: 28

Number of tables: 3

Number of figures: 2

Trial Registration: NCT00874341, ClinicalTrials.gov

ABSTRACT

CONTEXT: In observational studies low serum 25-hydroxyvitamin D (25-OHD) concentration is associated with an increased risk of type 2 diabetes mellitus (DM). Increasing serum 25-OHD may have beneficial effects on insulin resistance or beta-cell function. Cross-sectional studies utilising sub-optimal methods for assessment of insulin sensitivity and serum 25-OHD concentration provide conflicting results.

OBJECTIVE: This study examined the relationship between serum 25-OHD concentration and insulin resistance in healthy overweight individuals at increased risk of cardiovascular disease, using optimal assessment techniques.

METHODS: 92 subjects (mean age 56.0, SD 6.0 years), who were healthy but overweight (mean BMI 30.9, SD 2.3 kg/m²) underwent assessments of insulin sensitivity (two-step euglycaemic hyperinsulinaemic clamp, HOMA2-IR), beta-cell function (HOMA2%B), serum 25-OHD concentration and body composition (DEXA).

RESULTS: Mean total 25-OHD concentration was 32.2, range 21.8 – 46.6 nmol/L. No association was demonstrated between serum 25-OHD concentration and insulin resistance.

CONCLUSIONS: In this study using optimal assessment techniques to measure 25-OHD concentration, insulin sensitivity and body composition, there was no association between serum 25-OHD concentration and insulin resistance in healthy, overweight individuals at high risk of developing cardiovascular disease. This study suggests the documented inverse association between serum 25-OHD concentration

and risk of type 2 DM is not mediated by a relationship between serum 25-OHD concentration and insulin resistance.

INTRODUCTION

Vitamin D is a steroid hormone, which in active form binds to the vitamin D receptor. In observational studies low serum 25-hydroxyvitamin D (25-OHD) concentrations are associated with an increased risk of type 2 diabetes mellitus (DM) (1), a relationship that may be mediated via an effect on insulin resistance. Insulin resistance is defined as a reduced biological response to insulin (2). It is strongly associated with cardiovascular disease (CVD), is a characteristic feature of type 2 DM (3), and its attenuation may reduce the incidence of type 2 DM and CVD.

A variety of methods are used to assess insulin sensitivity, each having strengths and weaknesses, with the euglycaemic hyperinsulinaemic clamp technique considered the “gold standard” method of assessment (4). Most cross-sectional studies examining the relationship between 25-OHD concentration and insulin resistance have used surrogate markers of insulin resistance (1). Only four cross-sectional studies with mixed cohorts (and size, n=39, n=38, n=112, n=76) have used the euglycaemic hyperinsulinaemic clamp technique with all four demonstrating an inverse association with serum 25-OHD concentration (5-8).

Serum 25-OHD is the major circulating form of vitamin D and is widely considered to be the best indicator of vitamin D status (9). Different assay techniques for measuring 25-OHD result in variation of up to 20% above or below those obtained using the optimal technique of high-performance liquid chromatography and tandem mass spectrometry, which includes measurement of both the D2 and the D3 isoforms (10).

Obesity and insulin resistance are associated (11). The location of body fat is also important, with subjects with an android distribution of body fat having greater insulin resistance than those with a gynoid distribution (12). The relationship between obesity and serum 25-OHD concentration is complex. Serum 25-OHD concentrations decrease with increasing adiposity (13). Many of the studies examining the association between 25-OHD and insulin resistance have used body mass index (BMI) as an indirect measure of adiposity. In this study we measured body composition using a whole body Dual Energy X-ray Absorptiometry (DEXA) scan.

The aim of the present study was to examine the relationship between serum 25-OHD and insulin resistance in healthy but overweight individuals at increased risk of cardiovascular disease, using optimal techniques for the assessment of insulin sensitivity and 25-OHD concentration and controlling for body composition.

MATERIALS AND METHODS

Study overview

The data analysed in this study were collected at baseline from subjects in the “dose-response effect of fruit and vegetables on insulin resistance in healthy people who are overweight and at high risk of cardiovascular disease” trial. Ethical approval for this randomised controlled trial (RCT) was received from the Office for Research Ethics Committees Northern Ireland (ORECNI) and the study protocol was registered on ClinicalTrials.gov (trial registration no. NCT00874341). Methods and results have

been previously published (14). Data from 92 subjects who completed a two-step euglycaemic hyperinsulinaemic clamp were analysed.

Subject recruitment and screening

Between April 2009 and February 2011, subjects were recruited from the general population via press release, intranet advertisements within Belfast Health and Social Care Trust and Queen's University Belfast, and from hospital outpatient clinics. Interested participants were screened for eligibility. Inclusion criteria – CVD risk of $\geq 20\%$ over 10 years as defined by Joint British Societies' Guidelines on prevention of CVD in clinical practice (15), BMI ≥ 27 kg/m² and ≤ 35 kg/m², habitual fruit and vegetable intake ≤ 2 portions per day. Exclusion criteria - DM, established CVD, surgery within previous three months, aspirin use, psychiatric problems, taking medication known to affect nutrient metabolism, pregnant or lactating, excessive alcohol consumption, taking antioxidant supplements, food sensitivities that would interfere with a tolerance to fruit and vegetables, medical conditions or dietary restrictions that would substantially limit ability to complete the study requirements, following a weight loss diet, unwillingness or inability to modify current diet, women of childbearing age not taking the contraceptive pill.

Study assessments

Assessments were performed at the Regional Centre for Endocrinology and Diabetes, Royal Victoria Hospital, Belfast. Blood pressure (BP) was measured using an oscillometric Meditech ABPM-04 ambulatory BP system (P.M.S. (Instruments))

Ltd, Berkshire, UK). Height, weight, waist and hip circumference were recorded. Body composition was measured using a DEXA scan. All scans were performed by a trained radiographer using a Lunar Prodigy Pro DEXA scanner (GE Medical Systems, Madison, WI).

A two-step euglycaemic hyperinsulinaemic clamp combined with infusion of [3-3H] glucose was performed as previously described, aiming for a target plasma glucose concentration of 5.3 mmol/L (14). Briefly, a cannula was inserted into the left arm for infusions and in the right arm for samples. The right hand was placed in a temperature-controlled plexiglass box (55°C) to arterialise the venous blood. During a 2-hour equilibration period (-120 – 0 mins), a primed continuous infusion of [3-3H] glucose was administered. Initial tracer prime was adjusted, based on fasting plasma glucose concentration (16). A two-step sequential continuous infusion of insulin was administered: 0.4mU/kg/min (step 1, 0 - 120 mins), then 2.0 mU/kg/min (step 2, 120-240 mins). Plasma glucose concentration was measured at 5 minute intervals on a bedside analyser (Beckman glucose analyser 2, Beckman RIIC Ltd., High Wycombe, UK) and maintained at the target plasma glucose concentration of 5.3 mmol/L by alternating rates of exogenous 20% glucose infusion. Exogenous glucose was pre-labelled with [3-3H] glucose to match the predicted basal glucose specific activity as described, with the modification that the primed continuous tracer infusion was decreased to 50% of initial rate after 20 mins and to 25% of initial rate after 140 mins of insulin infusion (to maintain tracer steady state)(16).

Insulin resistance was assessed using exogenous glucose infusion rate (GIR) required to maintain euglycaemia corrected for body weight and for fat free body mass. The isotope dilution method was used to allow measurement of endogenous

glucose production (EGP), rate of appearance of glucose in the peripheral circulation (Ra), and rate of disappearance or whole body uptake of glucose (Rd).

Laboratory analysis

All samples were collected on the day of the euglycaemic hyperinsulinaemic clamp. All samples were processed and stored at -80°C within 2 hours of collection. Assays were standardised against appropriate National Institute of Standards and Technology (NIST) control materials. Serum insulin was measured by enzyme linked immunosorbent assay (ELISA) (Abbot IMx; Abbott Laboratories, Berkshire, U.K.). Plasma glucose was measured using an automated glucose oxidase method using a Beckman glucose analyser 2 (Beckman RIIC Ltd., High Wycombe, UK). The average of a minimum of 3 samples (4 were collected on each subject) of fasting glucose and insulin were used to calculate HOMA-IR and HOMA%B score (17). HOMA2-IR and HOMA2%B were calculated using an online calculator, accessed via the University of Oxford Diabetes Trials Unit website (www.dtu.ox.ac.uk/homacalculator/index.php) (18). Lipids were measured using a standard enzymatic colorimetric assay and serum calcium, phosphate and albumin were measured using a photometric method on an automated Roche Cobas 8000 Modular system biochemical analyser (Roche Diagnostics, West Sussex, UK). Serum concentrations of 25-OHD_{2&3} were measured by means of liquid chromatography tandem mass spectrometry (LC-MS/MS) and results presented as total 25-OHD (19).

Statistical methods

All statistical analyses were carried out using SPSS for Windows version 17.0 (SPSS Inc, Chicago, IL). Normally distributed continuous variables are summarised as arithmetic mean +/- standard deviation. Categorical variables are reported as percentages. Skewed variables were logarithmically transformed to allow parametric analysis and are summarised using the geometric mean and interquartile range. In the entire cohort the degree of linear association between total 25-OHD concentration and selected variables was assessed using Pearson's correlation coefficients. The study size was sufficient to give 90% power to detect as statistically significant ($p < 0.05$, two-tailed) a correlation coefficient of 0.33 or greater. To adjust for the possibility of confounding factors, including body composition, partial correlation analysis was performed. The cohort of 92 subjects was divided into three sub-groups based on 25-OHD concentration (deficient (25-OHD concentration < 25 nmol/L), insufficient (25-OHD concentration $25 - 50$ nmol/L) and adequate (25-OHD concentration > 50 nmol/L), using UK Department of Health criteria for adequacy of vitamin D (20)). The three sub-groups were compared using one way analysis of variance (ANOVA) for continuous variables and chi-squared test for categorical variables.

RESULTS

Subject characteristics

Subject characteristics are summarised in Table 1. As expected from the inclusion and exclusion criteria participants were overweight or obese (mean BMI 30.9, SD 2.3 kg/m²) with mild to moderate hypertension (mean systolic BP 141, SD 15 mmHg, with 29% on antihypertensive therapy) and dyslipidaemia (mean total cholesterol 5.5,

SD 1.1 mmol/L and mean total cholesterol:HDL cholesterol ratio 4.4, range 3.6 – 5.2 with 35% on pharmaceutical lipid-lowering therapy). Forty-two percent had prediabetes (21). Mean HbA1c was 36.3 mmol/mol, SD 3.3 mmol/mol (5.5%, SD 0.3%).

Mean serum total 25-OHD concentration was 32.2, range 21.8 – 46.6 nmol/L, almost entirely in the form of 25-OHD₃. Serum 25-OHD₂ concentration was highly skewed with 84 of 92 participants having a measured concentration below the assay detection level (0.895 nmol/L) and hence we report total 25-OHD concentration. Using UK Department of Health criteria for adequacy of vitamin D, 33% (n=30) were classed as deficient with measured serum vitamin D concentration less than 25 nmol/L, 47% (n=43) insufficient with serum vitamin D concentration between 25 and 50 nmol/L and only 20% (n=19) were classed as having adequate serum vitamin D (concentration greater than 50 nmol/L) (20). Serum calcium, phosphate, alkaline phosphatase and albumin concentrations for the 92 subjects were all within laboratory normal reference ranges.

Insulin sensitivity was assessed using the 2-step euglycaemic hyperinsulinaemic clamp technique. Plasma glucose concentration was successfully maintained at the target concentration (5.3 mmol/L) with a coefficient of variation of less than 5%. Steady state serum insulin concentration and glucose infusion rate were successfully achieved during the plateau phases (between 90 and 120 minutes for step 1 and between 210 and 240 minutes for step 2).

Association between serum 25-OHD concentration and insulin resistance

There was no statistically significant correlation between serum 25-OHD concentration and any measure of insulin sensitivity (including correction of glucose infusion rate for fat free mass, results not shown) (Table 2). Mean HOMA-IR was 1.8 (1.3 – 2.5) and mean HOMA2-IR was 1.0 (0.7 – 1.3). Correlation coefficients were not statistically significant. To examine the possibility of a non-linear association between serum 25-OHD concentration and measures of insulin sensitivity data were viewed graphically via scatter-plots with no association at any points along the range of serum 25-OHD concentrations (Figure 1 and 2).

To examine the possibility of confounding, partial correlation analysis was performed (Table 3). No significant correlation between serum total 25-OHD concentration and exogenous glucose infusion rate required to maintain euglycaemia during the insulin infusion during step 1 (GIR step 1) or during step 2 (GIR step 2) was demonstrated after controlling for potential confounding variables (gender, season of sampling, age, weight, BMI, waist circumference, hip circumference, waist:hip ratio, total body fat percentage, android fat percentage, gynoid fat percentage, fasting plasma glucose concentration and fasting serum insulin concentration).

25-OHD concentration subgroups

To examine the possibility of a threshold effect the cohort of 92 subjects was divided into three subgroups based on serum 25-OHD concentration (Table 1). The subgroups were compared using one way ANOVA and no statistically significant trend was demonstrated with increasing serum 25-OHD concentration.

Association between serum 25-OHD concentration and beta-cell function

Beta-cell function was assessed using HOMA%B and HOMA2%B with geometric mean values of 61.2 (44.7 – 83.4%) and 66.1 (53.1 – 82.5%) respectively. There were no significant correlations between serum 25-OHD concentration and measures of beta-cell function. Comparing the three subgroups based on serum 25-OHD concentration, one way ANOVA demonstrated no significant between group difference or trend (results not shown). One subject had a serum 25-OHD concentration greater than 100 nmol/L, and repetition of all analyses after exclusion of this subject did not alter the negative findings.

DISCUSSION

Using optimal techniques to assess insulin sensitivity and serum 25-OHD concentration, this cross-sectional analysis demonstrated no relationship between serum 25-OHD concentration and insulin resistance in healthy, overweight individuals at high risk of cardiovascular disease.

Findings with respect to insulin resistance are not inconsistent with previous reviews and meta-analyses (1,22,23). However, our results are largely discordant with the four cross-sectional studies which used the euglycaemic hyperinsulinaemic clamp technique to examine the relationship between serum 25-OHD concentration and insulin resistance, with all four reporting an inverse association (5-8). Muscogiuri et al demonstrated an inverse association between serum 25-OHD concentration and insulin resistance, which was no longer significant after correction for BMI.

Compared to our study, the cohort was smaller (39 compared to 92 subjects), younger (mean age 41 compared to 56 years) and with differing HOMA-IR (mean

HOMA-IR 2.6 compared to 1.8). Subjects had a similar BMI (mean 30.1 +/- 5.4 kg/m² compared to 30.9 +/- 2.3 kg/m²) and both studies included participants with a range of glucose tolerance. However, the Muscogiuri et al study included several subjects with very high BMI (5 greater than 40 kg/m²). It is possible that the significant correlation in this small cohort between serum 25-OHD concentration and insulin resistance was driven by these individuals with a very high BMI (5). The same group also reported an inverse relationship between insulin resistance and serum 25-OHD concentration in 38 premenopausal women with polycystic ovarian syndrome. Again, after correction for total fat mass (assessed using DEXA) this association was no longer statistically significant (6). In a study of 112 postmenopausal French-Canadian women, Morisset et al reported a correlation between insulin resistance and serum 25-OHD concentration. In this cohort, the association between serum 25-OHD concentration and insulin resistance was independent of glucose tolerance status (numbers are not reported), but did not adjust for body composition, which had been assessed as part of the study (7). In a cohort of 76 women (42 with and 34 without polycystic ovarian syndrome) Joham et al reported no association between serum 25-OHD and insulin resistance in the total group after adjustment for adiposity and age. However in the group of 42 with polycystic ovarian syndrome they report an association that remains significant after adjustment for age and body fat percentage (8). These four studies used a chemiluminescent assay to measure serum 25-OHD concentration, rather than the optimal technique of high-performance liquid chromatography and tandem mass spectrometry utilised in our study. We suggest that the well-defined cohort of healthy, overweight subjects at increased cardiovascular disease risk analysed in our study, may not be comparable to the cohorts analysed in these studies.

In contrast to several large studies, we demonstrated no association between insulin sensitivity measured by HOMA-IR and serum 25-OHD concentration (1). HOMA-IR may be altered by small perturbations in measured plasma glucose and serum insulin concentrations and should be based upon the mean of three separate samples (17). All samples in our study were processed in a single laboratory and were based on a mean of at least three fasting measurements. It is not clear if such stringent criteria were adhered to in other studies. It has been recommended that the computer modelling technique (HOMA2-IR) is used. We also demonstrated no association between serum 25-OHD concentration and HOMA2-IR (18).

We demonstrate no association between serum 25-OHD concentration and beta-cell function (HOMA%B and HOMA2%B). A recent systematic review included five randomised controlled trials and found no significant effect of vitamin D supplementation on HOMA%B (23). However, studies using the more intensive hyperglycaemic clamp method have demonstrated a significant improvement in beta-cell function in response to an increase in serum 25-OHD concentration (24,25).

There are plausible mechanisms of association. Vitamin D exerts its effects via the vitamin D receptor which is found on many cells including beta-cells. The enzyme 1α -hydroxylase which forms the active form of vitamin D is also located in beta-cells and vitamin D may mediate increased insulin secretion via increases in intracellular calcium through the phosphoinositide/protein kinase C pathway and facilitating calcium entry via calcium channels (26).

Low serum 25-OHD concentrations are associated with an increased risk of development of type 2 DM (1). Based on the accumulated evidence from this

analysis and others (1), it seems unlikely that this relationship can be explained by an association between serum 25-OHD concentration and insulin resistance.

Our study has a number of strengths. It is the only cross-sectional analysis examining the relationship between serum 25-OHD concentration and insulin resistance using optimal techniques to assess insulin sensitivity, body composition and serum 25-OHD concentration. Subjects were well characterised with anthropometry and accurate assessment of cardiovascular disease risk factors. All subjects had a DEXA scan to assess adiposity and body fat distribution.

Plasma parathyroid hormone (PTH) concentration was not measured in the present study but is a possible confounder of an association between serum 25-OHD concentration and insulin resistance. There is an increased prevalence of type 2 DM in patients with primary hyperparathyroidism (27) and reduced insulin secretion and reduced insulin sensitivity have both been demonstrated in subjects with elevated plasma PTH concentrations (28).

Whilst our study is large with respect to the euglycaemic hyperinsulinaemic clamp, studies using other techniques to assess insulin sensitivity may use larger numbers of subjects increasing statistical power. Our study size was sufficient to give 90% power to detect as statistically significant ($p < 0.05$, two-tailed) a correlation coefficient of 0.33 or greater.

We used a surrogate assessment of beta-cell function (HOMA%B and HOMA2%B) which reflects only changes in the fasting state. These techniques are arguably less sensitive than more intensive methods (such as the hyperglycaemic clamp) and we suggest that conclusions on beta-cell function based on our results are limited..

This study demonstrated no relationship between serum 25-OHD concentration and insulin resistance in healthy, overweight individuals at high risk of developing cardiovascular disease. We suggest it is unlikely there is a significant association between serum 25-OHD concentration and insulin resistance. It remains possible that the documented association between serum 25-OHD concentration and risk of type 2 DM is mediated by an effect of serum 25-OHD concentration on beta-cell secretory response, rather than insulin resistance. A prospective, randomised placebo controlled trial of adequate duration on high risk individuals given sufficient vitamin D supplementation, utilising the euglycaemic hyperinsulinaemic clamp technique to assess insulin sensitivity and liquid chromatography tandem mass spectrometry to measure 25-OHD, with assessments performed before and after the intervention, is required to assess causality of these possible associations.

References:

(1) Wallace, I.R., Wallace, H.J., McKinley, M.C., Bell, P.M., Hunter, S.J. (2015) Vitamin D and insulin resistance. *Clinical endocrinology*, doi: 10.1111/cen.12760.

(2) Kahn, C.R. (1978) Insulin resistance, insulin insensitivity, and insulin unresponsiveness: a necessary distinction. *Metabolism: Clinical & Experimental*, 27, 1893-1902.

(3) Wilson, P.W., D'Agostino, R.B., Parise, H., Sullivan, L., Meigs, J.B. (2005) Metabolic syndrome as a precursor of cardiovascular disease and type 2 diabetes mellitus. *Circulation*, 112, 3066-3072.

(4) Muniyappa, R., Lee, S., Chen, H., Quon, M.J. (2008) Current approaches for assessing insulin sensitivity and resistance in vivo: advantages, limitations, and appropriate usage. *American Journal of Physiology - Endocrinology & Metabolism*, 294, E15-26.

(5) Muscoqiuri, G., Sorice, G.P., Prioletta, A., Policola, C., Della Casa, S., Pontecorvi, A., et al (2010) 25-Hydroxyvitamin D concentration correlates with insulin-sensitivity and BMI in obesity. *Obesity*, 18, 1906-1910.

(6) Muscoqiuri G, Policola C, Prioletta A, Sorice G, Mezza T, Lassandro A, et al (2012) Low levels of 25(OH)D and insulin-resistance: 2 unrelated features or a cause-effect in PCOS?. *Clinical Nutrition*, 31, 476-480.

(7) Morisset, A.S., Tardio, V., Weisnagel, J., Lemieux, S., Bergeron, J., Gagnon, C. (2015) Associations between serum 25-hydroxyvitamin D, insulin sensitivity, insulin secretion, beta-cell function according to glucose tolerance status. *Metabolic Syndrome & Related Disorders*, doi: 10.1089/met.2014.0159.

(8) Joham, A.E., Teede, H.J., Cassar, S., Stepto, N.K., Strauss, B.J., Harrison, C.L., et al (2015) Vitamin D in polycystic ovary syndrome: relationship to obesity and insulin resistance. *Molecular Nutrition & Food Research*, DOI: 10.1002/mnfr.201500259.

(9) Seamans, K.M., Cashman, K.D. (2009) Existing and potentially novel functional markers of vitamin D status: a systematic review. *Am.J.Clin.Nutr.*, 89, 1997S-2008S.

(10) Tahsin-Swafiri, S., Bianco-Navarro, I., Perez-Sacristan, B., Millan, I., Granado-Lorencio, F. (2012) The prevalence of vitamin deficiency in clinical practice is assay-dependent. *Clinical Nutrition*, 31, 1011-1014.

(11) Ferrannini, E., Natali, A., Bell, P., Cavallo-Perin, P., Lalic, N., Mingrone, G. (1997) Insulin resistance and hypersecretion in obesity. European Group for the Study of Insulin Resistance (EGIR). *J.Clin.Invest.*, 100, 1166-1173.

(12) Gautier, J.F., Mourier, A., de Kerviler, E., Tarentola, A., Bigard, A.X., Villette, J.M., et al (1998) Evaluation of abdominal fat distribution in noninsulin-dependent diabetes mellitus: relationship to insulin resistance. *Journal of Clinical Endocrinology & Metabolism*, 83, 1306-1311.

(13) Forsythe, L.K., Livingstone, M.B., Barnes, M.S., Horigan, G., McSorley, E.M., Bonham, M.P., et al (2012) Effect of adiposity on vitamin D status and the 25-hydroxycholecalciferol response to supplementation in healthy young and older Irish adults. *Br.J.Nutr.*, 107, 126-134.

(14) Wallace IR, McEvoy CT, Hunter SJ, Hamill LL, Ennis CN, Bell PM, et al (2013) Dose-response effect of fruit and vegetables on insulin resistance in people at high risk of cardiovascular disease: a randomized controlled trial. *Diabetes Care*, 36, 3888-3896.

(15) British Cardiac, S., British Hypertension, S., Diabetes, U.K., Heart, U.K., Primary Care Cardiovascular, S., Stroke, A. (2005) JBS 2: Joint British Societies' guidelines on prevention of cardiovascular disease in clinical practice. *Heart*, 91, 1-52.

(16) Neely, R.D., Rooney, D.P., Atkinson, A.B., Sheridan, B., Ennis, C.N., Trimble, E.R., et al (1990) Underestimation of glucose turnover determined using [6-3H]glucose tracer in non-steady state. The role of a tritiated tracer impurity. *Diabetologia*, 33, 681-687.

(17) Matthews, D.R., Hosker, J.P., Rudenski, A.S., Naylor, B.A., Treacher, D.F., 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.

(18) Levy, J.C., Matthews, D.R., Hermans, M.P. (1998) Correct homeostasis model assessment (HOMA) evaluation uses the computer program. *Diabetes Care*, 21, 2191-2192.

(19) Bennett SE, McPeake J, McCance DR, Manderson JG, Johnston P, McGalliard R, et al (2013) Maternal vitamin D status in type 1 diabetic pregnancy: impact on neonatal vitamin D status and association with maternal glycaemic control. *PLoS ONE [Electronic Resource]*, 8, e74068.

(20) UK Department of Health (1998) Nutrition and bone health: with particular reference to calcium and vitamin D. Report of the Subgroup on Bone Health, Working Group on the Nutritional Status of the Population of the Committee on Medical Aspects of the Food Nutrition Policy. *Reports on Health & Social Subjects*, 49.

(21) American Diabetes Association. (2013) Diagnosis and classification of diabetes mellitus. *Diabetes care*, 36, S67--S74.

(22) Autier, P., Boniol, M., Pizot, C., Mullie, P. (2014) Vitamin D status and ill health: a systematic review. *The Lancet Diabetes and Endocrinology*, 2, 76-89.

(23) Seida, J., Mitri, J., Colmers, I., Majumdar, S., Davidson, M., Edwards, A., et al (2014) Effect of vitamin D3 supplementation on improving glucose homeostasis and preventing diabetes: a systematic review and meta-analysis. *Journal of Clinical Endocrinology & Metabolism*, 99, 3551-3560.

(24) Karnchanasorn R, Ou HY, Chiu KC (2012) Plasma 25-hydroxyvitamin D levels are favorably associated with beta-cell function. *Pancreas*, 41, 863-868.

(25) Kampmann U, Mosekilde L, Juhl C, Moller N, Christensen B, Rejnmark L, et al (2014) Effects of 12 weeks high dose vitamin D3 treatment on insulin sensitivity, beta cell function, and metabolic markers in patients with type 2 diabetes and vitamin D insufficiency - a double-blind, randomized, placebo-controlled trial. *Metabolism: Clinical & Experimental*, 63, 1115-1124.

(26) Billaudel, B.J., Boursol, P.M., Sutter, B.C., Faure-Dussert, A.G. (1995) Regulatory effect of 1,25-dihydroxyvitamin D3 on insulin release and calcium handling via the phospholipid pathway in islets from vitamin D-deficient rats. *J.Endocrinol.Invest.*, 18, 673-682.

(27) Taylor, W.H. (1991) The prevalence of diabetes mellitus in patients with primary hyperparathyroidism and among their relatives. *Diabetic Med.*, 8, 683-687.

(28) Chiu, K.C., Chuang, L.M., Lee, N.P., Ryu, J.M., McGullam, J.L., Tsai, G.P., et al (2000) Insulin sensitivity is inversely correlated with plasma intact parathyroid hormone level. *Metabolism: Clinical & Experimental*, 49, 1501-1505.

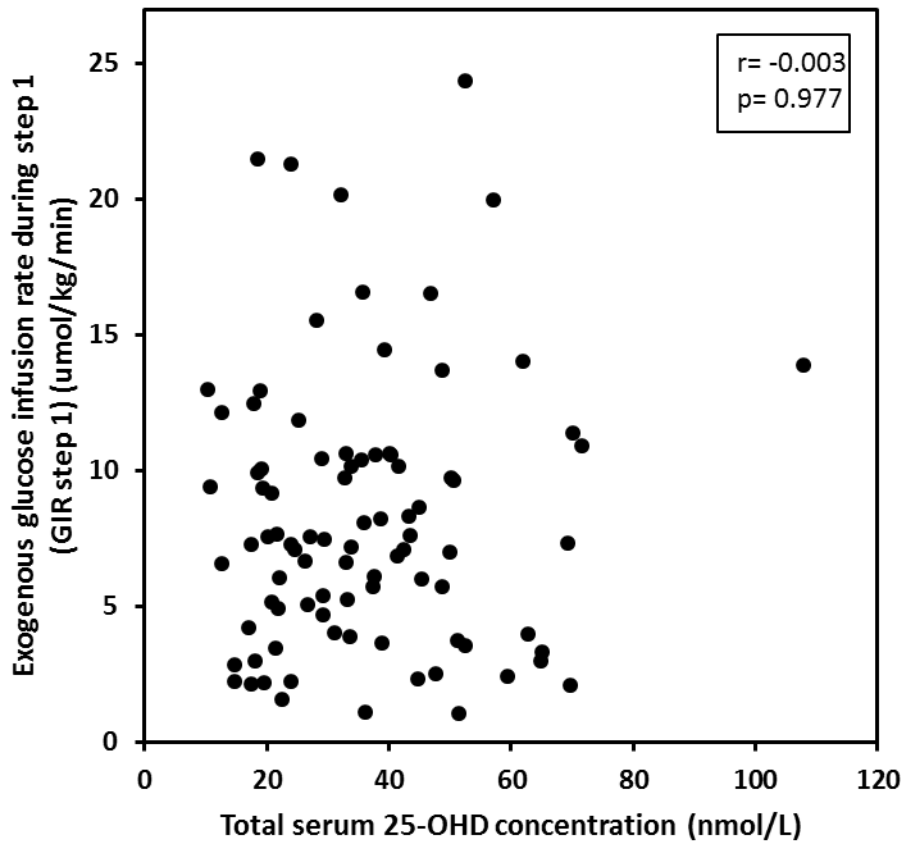


Figure 1: Scatterplot showing GIR step 1 against total vitamin D (25-OHD) concentration (n=89)

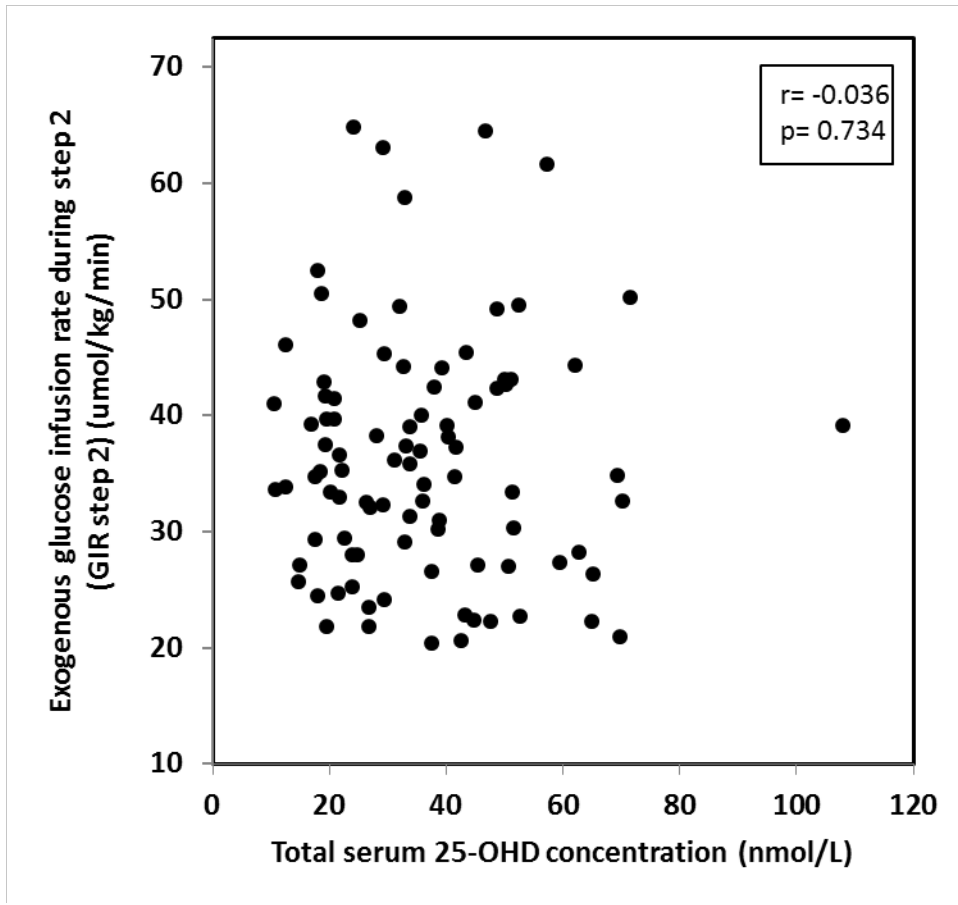


Figure 2: Scatterplot showing GIR step 2 against total vitamin D (25-OHD) concentration (n=92)

Table 1: Subject characteristics (total group (n=92) and subgroups as defined by 25-OHD concentration

	Total group (n=92)	Deficient (<25 nmol/L) (n=30 (33%))	Insufficient (25 - 50 nmol/L) (n=43 (47%))	Adequate (>50 nmol/L) (n=19 (20%))
Total 25-OHD concentration (nmol/L)	32.2 (21.8 – 46.6)	18.5 (17.5 – 21.8)	35.9 (31.1 – 41.8)	60.5 (51.3 – 69.4)
Gender (%), (n)	Male 64% (59)	Male 67% (20)	Male 63% (27)	Male 63% (12)
Systolic Blood Pressure (mmHg)	141 (15)	143 (13)	139 (16)	143 (18)
Diastolic Blood Pressure (mmHg)	93 (11)	92 (10)	93 (12)	95 (10)
BMI (kg/m²)	30.9 (2.3)	31.1 (2.7)	30.4 (2.1)	31.8 (2.1)
Fasting plasma glucose (mmol/L)	5.5 (0.6)	5.9 (0.5)	5.8 (0.4)	5.9 (0.5)
Fasting serum insulin (mU/L)	7.1 (5.2 – 9.4)	7.8 (5.8 – 9.9)	6.7 (4.8 – 9.3)	6.7 (5.4 – 8.8)

GIR corrected for absolute weight Step 1 (µmol/kg/min)	6.6 (4.1 – 10.6)	6.2 (3.2 – 10.0)	7.3 (5.6 -10.6)	5.9 (3.3 -11.7)
GIR corrected for absolute weight Step 2 (µmol/kg/min)	34.7 (28.0 – 42.4)	34.7 (28.0 – 41.1)	34.9 (29.1 – 42.4)	34.2 (27.0 – 43.1)
EGP Basal (µmol/kg/min)	10.4 (1.9)	10.5 (2.2)	10.4 (1.9)	10.5 (1.3)
EGP Step 1 (µmol/kg/min)	5.6 (2.3)	5.4 (1.9)	5.2 (2.5)	7.0 (2.1)
EGP Step 2 (µmol/kg/min)	1.8 (1.5 – 5.6)	1.2 (1.0 – 4.7)	2.1 (1.7 – 5.5)	2.3 (2.7 – 8.0)
Rd Basal (µmol/kg/min)	10.4 (1.8)	10.4 (2.1)	10.3 (1.9)	10.4 (1.1)
Rd Step 1 (µmol/kg/min)	13.2 (10.5 – 15.8)	12.7 (9.8 – 15.3)	13.0 (10.5 – 15.3)	14.6 (10.6 – 19.5)
Rd Step 2 (µmol/kg/min)	39.9 (11.1)	38.9 (9.8)	40.6 (11.6)	40.1 (12.4)

$p_{\text{between group}}$ and p_{trend} were non-significant ($p > 0.05$)

Table 2: Correlation coefficients (Pearson (r)) between total 25-OHD and assessments of insulin resistance and beta-cell function

	Whole group (n=92)	95% CI
GIR corrected for	-0.00	-0.21 - 0.26

absolute weight Step 1		
GIR corrected for absolute weight Step 2	-0.00	-0.28 – 0.14
EGP Basal	0.05	-0.16 – 0.32
EGP Step1	0.19	-0.01 – 0.37
EGP Step 2	0.14	-0.07 – 0.34
Rd Basal	0.02	-0.19 – 0.26
Rd Step 1	0.12	-0.12 – 0.33
Rd Step 2	0.03	-0.22 – 0.21
Ra Basal	0.05	-0.17 – 0.32
Ra Step 1	0.14	-0.09 – 0.34
Ra Step 2	0.01	-0.25 – 0.18

All correlation coefficients were non-significant ($p>0.05$)

Table 3: Partial correlation analysis for total 25-OHD concentration and GIR step 1 and for total 25-OHD concentration and GIR step 2

	Total 25-OHD concentration and GIR step 1		Total 25-OHD concentration and GIR step 2	
	Correlation Coefficient (r)	95% CI	Correlation Coefficient (r)	95% CI
-	-0.00	-0.21 – 0.26	-0.00	-0.28 – 0.14
Gender	0.01	-0.22 – 0.21	-0.05	-0.25 – 0.13
Age	-0.01	-0.22 – 0.22	-0.06	-0.25 – 0.15

Weight	0.03	-0.20 – 0.25	-0.02	-0.25 – 0.18
BMI	0.01	-0.22 – 0.26	-0.05	-0.25 – 0.16
Waist Circumference	0.03	-0.20 – 0.25	-0.02	-0.22 – 0.17
Hip Circumference	-0.00	-0.23 – 0.22	-0.06	-0.27 - 0.13
Waist Hip Ratio	0.02	-0.22 – 0.25	-0.05	-0.25 – 0.15
Total Body Fat Percentage	0.00	-0.21 – 0.24	-0.06	-0.26 – 0.13
Android Fat Percentage	-0.01	-0.22 – 0.24	-0.08	-0.26 – 0.12
Gynoid Fat Percentage	0.01	-0.21 – 0.22	-0.05	-0.25 – 0.13
Fasting Glucose	-0.01	-0.25 – 0.22	-0.07	-0.27 – 0.11
Fasting Insulin	-0.11	-0.34 – 0.13	-0.19	-0.38 – 0.01

All correlation coefficients were non-significant ($p>0.05$)