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# Changes in metabolic indices in response to whole blood donation in male subjects with normal glucose tolerance

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ABSTRACT

**Objectives:** Previous studies have investigated the impact of venesection upon different metabolic indices in patients with various conditions (e.g., type 2 diabetes and iron overload). We aimed to investigate the changes on different metabolic indices including glycemic, iron, lipids and inflammatory markers at different time points after blood donation in male subjects with normal glucose tolerance.

**Design and methods:** 42 male subjects were recruited to the study. Glucose tolerance was assessed by oral glucose tolerance test before (visit A) and after the blood donation (1 day, visit B; 1 week, visit C; 3 weeks, visit D; and 3 months, visit E). Fasting glucose, HbA1c, insulin, lipids, uric acid, C-reactive protein, iron stores and insulin resistance (HOMA-IR, ISI-gly) indices were measured. A repeated measures ANOVA was used for comparisons of quantitative variables between different visits.

**Results:** All subjects had normal glucose tolerance according to WHO criteria. Fasting glucose, insulin and HOMA-IR were significantly higher (~2%, $p < 0.05$; ~21%, $p < 0.01$; and ~11%, $p < 0.05$ respectively) at visit B following donation. At visit D, the mean ± SE for HbA1c (5.28 ± 0.06%) was significantly lower with a difference in percentage of ~− 3% and $p < 0.05$ compared to visit A (5.44 ± 0.06%). Ferritin decreased significantly at visits B, C, D and E (~− 8%, $p < 0.01$, ~− 24%, $p < 0.001$, ~− 39%, $p < 0.001$ and ~− 29%, $p < 0.01$ respectively), when compared to visit A.

**Conclusions:** At different time points after blood donation, glycemic status and iron stores are affected significantly in male blood donors with normal glucose tolerance. The changes were particularly evident three weeks after donation. Hence, the interpretation of these parameters in male blood donors needs to take this into account, and the mechanisms resulting in these effects need to be clarified.



## 1. Introduction

Regular blood donation appears to be associated with a reduced risk of developing type 2 diabetes and cardiovascular disease [1,2], including a lower risk of acute myocardial infarction [3]. In patients with type 2 diabetes with high serum ferritin concentrations, a significant improvement in insulin resistance (IR) was observed following blood donation compared to controls [4]. In a recent report a lower prevalence of diabetes was recorded among frequent blood donors [5]. Other findings support the notion that a reduction in body iron stores enhances insulin sensitivity, even in "iron sufficient" individuals. However, no previous studies have investigated variation in metabolic parameters of glucose tolerance, lipids, inflammatory and iron stores in normal individuals at different time points following blood donation. In this study we have investigated changes in these indices over the short and longer term following blood donation in male subjects with normal glucose tolerance.

## 2. Subjects and methods

The individuals recruited were 42 males. Subjects were excluded if they were being treated with oral hypoglycaemic medications (e.g., metformin), insulin or other drugs likely to modify insulin sensitivity e.g., steroids [6]. In addition, subjects were excluded if test results suggested abnormal glucose tolerance, abnormal iron stores or if they had

*Abbreviations:* OGTT, oral glucose tolerance test; HOMA-IR, homeostasis model assessment insulin resistance; ISI-gly, insulin sensitivity index-glycaemia; NGT, normal glucose tolerance; IR, insulin resistance; HbA1c, haemoglobin A1c.

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a current acute illness, cancer, acute myocardial infarction, symptoms of cardiovascular disease, malabsorption or intestinal obstruction. Subjects were also excluded if less than six months had passed since their last donation or if there were other factors which contraindicated blood donation. The study was confined to male subjects in order to avoid the need to consider possible confounding effects of hormonal cycle variation on metabolic indices, which would be present in most female blood donors. All subjects gave informed consent for participation and the study was approved by King Abdullah International Medical Research Center Ethics Committee, Jeddah, Saudi Arabia (Study number RCJ0611-183).

Body mass index (BMI; kg/m$^2$) and blood pressure (BP; mmHg) were measured at the first visit (visit A). A 75 g oral glucose tolerance test (OGTT) was performed on each subject between 8 and 10 am during this visit. Fasting blood samples were collected into ethylenediaminetetra-acetic acid (EDTA), fluoride oxalate (grey top), and plain tubes. The glucose load was ingested over 5 min. After 2 h further blood samples were collected into plain and grey top tubes. Within 10 min of collection, plasma and serum were separated by centrifugation (3000 ×*g*) for 10 min. Plasma glucose was measured immediately. Serum specimens for specialised tests namely insulin, lipids (total cholesterol, HDL-C, calculated LDL-C, triglyceride), iron, ferritin and transferrin were stored at −80 °C until analysis. EDTA-anticoagulated whole blood was collected for glycated haemoglobin (HbA1c) and complete blood count (CBC) measurement. After the OGTT was completed, participants were given lunch. Once the results of the OGTT were available the glycaemic status of subjects was categorized according to WHO criteria. If a normal glucose tolerance was confirmed, blood donation (450 mL) was undertaken later the same day by standard protocol at the blood bank donation area.

Subjects returned for a second visit (visit B) 24 h after the blood donation. The second OGTT was carried out as described at the first visit but without further blood donation. A week later a third OGTT was performed (visit C) and two weeks later a fourth OGTT was performed (visit D). Three months after the fourth visit 10 subjects attended for a fifth visit (visit E). The schedule of visits is summarised in Fig. 1. Subjects were not taking any medication or supplements. They were instructed to maintain their usual lifestyle and food intake before and between visits, because any changes in these factors could potentially affect their metabolic indices independently of blood donation. Glycaemic status of the subjects was classified according to WHO criteria [7].

### 2.1. Assays

Routine laboratory analyses were carried out on an Architect autoanalyser (Abbott, USA).

The laboratory at King Abdulaziz Medical City is accredited by the College of American Pathologists. It participates in a rigorous programme of internal quality control and external quality assessment. Glucose was assayed by the hexokinase method and urate by a uricase method. C-reactive protein (CRP) was measured by immunoturbidimetry. Cholesterol was measured by an enzymatic method using cholesterol esterase [8], HDL-cholesterol by a direct method using a colorimetric endpoint based on Trinder reaction [9] and triglycerides by a method based on the Fossati three-step enzymatic reaction [10]. LDL-cholesterol was estimated by calculation using the Friedewald formula [11]. Iron and UIBC were measured based on the principle of hydroxylamine hydrochloride and ferene methods respectively. HbA1c analysis was by high pressure liquid chromatography on a TOSOH Bioscience, Inc instrument (South San Francisco, CA). Insulin and ferritin were measured by chemiluminescent immunoassay CMIA (Architect, i1000).

### 2.2. Indices of insulin resistance

Insulin resistance and β-cell activity indices were calculated as shown in Table 1. The use of these indices is discussed in detail elsewhere [12,13].

### 2.3. Statistics

Descriptive results of continuous variables are expressed as mean ± SD or SE. Before statistical analysis, normal distribution and homogeneity of the variances were evaluated using Levene's test, and the variables log transformed if necessary. Comparisons of quantitative variables were by paired *t* test. Repeated measures ANOVA was performed as a separate analysis when necessary.

## 3. Results

All 42 subjects had a normal glucose tolerance (NGT). The mean age (± SD) was 36 ± 7 years, body mass index (BMI) 27.3 ± 3.2 kg/m$^2$, and haemoglobin 16.0 ± 1.1 g/dL. BMI categorization of subjects was as follows: 28.6% were normal, 54.8% were overweight, and 16.6% obese. Changes in metabolic indices before and after blood donation are shown in Table 2.

### 3.1. Glycaemic indices

Compared to the baseline level (5.3 ± 0.1 mmol/L) fasting plasma glucose was significantly higher (5.4 ± 0.1; $p < 0.05$) at visit B; then decreased progressively to visit D (5.2 ± 0.1; $p < 0.01$) (Fig. 2A). Fasting glucose was significantly ($p < 0.0001$) changed from A to D visit by 22.1%. Post load (2 h) plasma glucose levels did not change significantly from baseline. A significant increase ($p < 0.01$, 21.3%) in fasting insulin was noticed at visit B when compared to visit A (Fig. 2B). Compared to the baseline level, mean HbA1c levels were slightly increased at visit B with a difference in percentage of 0.4% but at visit D the level was significantly decreased with a difference in percentage of −2.9% and $p < 0.05$ (Fig. 2C). The overall HbA1c level was significantly ($p = 0.005$) changed from A to D visits with a difference in percentage of 13.9%.

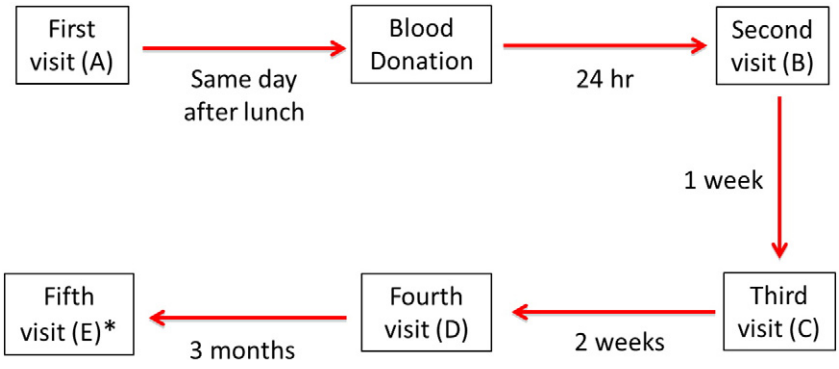


**Fig. 1.** Protocol for the involved subjects (n = 42) included in each visit and in between time interval. *10 subjects were involved only.