

RESEARCH ARTICLE

Open Access

Glucose challenge increases circulating progenitor cells in Asian Indian male subjects with normal glucose tolerance which is compromised in subjects with pre-diabetes: A pilot study

Abel A Nathan¹, Viswanathan Mohan², Subash S Babu³, Soumi Bairagi¹, Madhulika Dixit^{1*}

Abstract

Background: Haematopoietic stem cells undergo mobilization from bone marrow to blood in response to physiological stimuli such as ischemia and tissue injury. The aim of study was to determine the kinetics of circulating CD34⁺ and CD133⁺CD34⁺ progenitor cells in response to 75 g glucose load in subjects with normal and impaired glucose metabolism.

Methods: Asian Indian male subjects (n = 50) with no prior history of glucose imbalance were subjected to 2 hour oral glucose tolerance test (OGTT). 24 subjects had normal glucose tolerance (NGT), 17 subjects had impaired glucose tolerance (IGT) and 9 had impaired fasting glucose (IFG). The IGT and IFG subjects were grouped together as pre-diabetes group (n = 26). Progenitor cell counts in peripheral circulation at fasting and 2 hour post glucose challenge were measured using direct two-color flow cytometry.

Results: The pre-diabetes group was more insulin resistant ($p < 0.0001$) as measured by homeostasis assessment model (HOMA-IR) compared to NGT group. A 2.5-fold increase in CD34⁺ cells ($p = 0.003$) and CD133⁺CD34⁺ ($p = 0.019$) cells was seen 2 hours post glucose challenge in the NGT group. This increase for both the cell types was attenuated in subjects with IGT. CD34⁺ cell counts in response to glucose challenge inversely correlated with neutrophil counts ($\rho = -0.330$, $p = 0.019$), while post load counts of CD133⁺CD34⁺ cells inversely correlated with serum creatinine ($\rho = -0.312$, $p = 0.023$).

Conclusion: There is a 2.5-fold increase in the circulating levels of haematopoietic stem cells in response to glucose challenge in healthy Asian Indian male subjects which is attenuated in subjects with pre-diabetes.

Background

Haematopoietic stem cell (HSCs) characterized by surface expression of CD34, or CD133 or both exhibit extreme plasticity. Upon receipt of physiological stimuli these cells mobilize from bone marrow into peripheral circulation to mediate multi-lineage engraftment of organs of even non-haematopoietic origin such as skeletal muscle, central nervous system, heart and liver [1].

This pool of immature stem cells is capable of giving rise to progenitors of the cardiovascular system such as the endothelial progenitor cells (EPCs), cardiomyocytes and smooth muscle cell progenitors [2]. They follow circadian kinetics [3] and get mobilized in response to ischemia imparted by acute myocardial infarction (MI) [4]. Similarly during menstrual cycle EPCs follow rhythmic oscillations with maximal increase during ovulatory stage [5].

Abnormalities in carbohydrate metabolism form a continuum which progressively worsens as a subject transitions from NGT to IGT and from IGT to diabetes.

* Correspondence: mdixit@iitm.ac.in

¹Laboratory of Vascular Biology, Department of Biotechnology, Indian Institute of Technology Madras (IIT Madras), Chennai, India
Full list of author information is available at the end of the article

This progressive decline in carbohydrate metabolism parallels a concomitant decrease in vascular health and circulating bone marrow-derived vascular progenitor cells [6-11]. Both type 1- and type 2- diabetes are associated with significant reduction in circulating levels of CD34⁺ cells and EPCs [7,9-13]. Similarly a reduction in the circulating levels of EPCs is seen in IGT subjects [9,14]. However all these studies have looked at progenitor cell counts following overnight fasting and till date no information is available on kinetics of progenitor cells in response to glucose load. Thus the aims of this study were to determine 1) whether levels of CD34⁺, CD133⁺ and CD133⁺CD34⁺ progenitor cells increase in circulation in response to glucose load and if it does then 2) is the increase in progenitor counts altered in pre-diabetes subjects exhibiting early stages of compromised carbohydrate metabolism. The interest to better define the relationship between glucose load and progenitor kinetics, also stems from the observation that post-prandial glucose peaks are strongly correlated with increased cardiovascular risks [15,16].

Methods

Study Subjects

The study was approved by IIT Madras Institutional ethics committee in accordance with Declaration of Helsinki as revised in 2000 and as required by the Indian Council of Medical Research. Asian Indian male subjects undergoing their first metabolic examination with no prior history of glucose imbalance were recruited at Dr. Mohan's Diabetes Specialties Centre, a tertiary diabetes centre in Chennai, India. The following exclusion criteria were applied: diabetes, established CAD or CVD, smoking, acute infections or immunological disorders, cancer and recent surgery in the past 10 months. Following over-night fast, blood was drawn for quantification of circulating progenitor cells and for determination of their lipid, glucose, insulin and protein profiles. This was followed by 75 g OGTT test and blood was redrawn at 2 hours post glucose load for measurement of progenitor cells, glucose and insulin profiles.

Definitions and diagnostic criteria

NGT were defined as fasting plasma glucose (FPG) <5.5 mmol/l and 2 hour post load glucose <7.8 mmol/l as per WHO criteria [17]. Pre-diabetes group consisted of subjects with IGT, IFG and combined IGT/IFG. IGT was defined as 2 hour post-load plasma glucose \geq 7.8 mmol/l and <11.1 mmol/l as per WHO criteria. IFG was defined based on ADA criteria [17] as FPG \geq 5.5 mmol/l and <7 mmol/l and using WHO criteria as FPG \geq 6.1 mmol/l and <7 mmol/l. Among the total subjects recruited 24 were NGT, 17 were IGT and 9 subjects were IFG.

Anthropometric measurements

For all subjects the following parameters were recorded: age, body mass index (BMI), height, weight, waist to hip ratio (WHR) and blood pressure. BMI was calculated as weight in kilograms divided by height expressed in meters square. Blood pressure was measured in a sitting position for the right arm to the nearest mm of Hg using mercury sphygmomanometer (Diamond Deluxe; Pune, India). Two readings were taken with a 5 minute interval and the mean of the two was expressed as blood pressure.

Biochemical parameters

Plasma glucose by glucose oxidase-peroxidase method, serum cholesterol by cholesterol oxidase-peroxidase-4-aminophenazone method, serum triglycerides by glycerol phosphate oxidase peroxidase-4-aminophenazone method and HDL cholesterol via direct method (with polyethylene glycol-pretreated enzymes) were measured using the Hitachi-912 Autoanalyzer (Hitachi, Manheim, Germany). The intra- and inter-assay coefficient of variation (%CV) for these biochemical tests ranged between 3.1% and 7.6% respectively. LDL cholesterol values were derived using Friedewald formula. Insulin concentrations were estimated by enzyme-linked immunosorbent assay (Dako, Glostrup, Denmark). The intra- and inter-assay %CV for insulin measurements were 5.7% and 8.9% respectively. HbA_{1c} was measured by high-pressure liquid chromatography using the variant machine (BioRad, Hercules, California, USA). The intra- and inter-assay %CV of HbA_{1c} measurements was less than 10%. Insulin resistance was estimated using homeostasis assessment model of insulin resistance (HOMA-IR) calculated as fasting insulin (μ U/ml) X fasting glucose (mmol/l) divided by 22.5.

Quantification of circulating progenitor cells via Flow Cytometry

Peripheral blood progenitor cells were analyzed for surface expression of CD34 and CD133 through direct two-color flow cytometry. Peripheral blood was drawn each at fasting and 2 hr post-load conditions in EDTA vacutainers (BD Biosciences, USA). Prior to staining, samples were blocked with 5% fetal bovine serum and FcR reagent for 20 min. Samples were stained with CD133-PE and CD34-FITC antibodies (Miltenyi Biotech, Germany). Corresponding isotype control IgG1-PE and IgG2a-FITC (Miltenyi Biotech, Germany) antibodies were used. Following red cell lysis, a minimum of 5 lakh events were acquired and scored as per EUROSTAR guidelines [18] using FACS Calibur Flow cytometer (Becton Dickinson, USA). Data were processed using Flowjo software program (Version 7.6.1, Tree Star Inc., USA). The instrument was daily optimized by analyzing

the expression of peripheral blood leukocytes with anti-CD4 and anti-CD3 antibodies. The operator who acquired the FACS data was masked to the clinical status of the subjects. Following appropriate gating, CD34⁺, CD133⁺ and CD133⁺CD34⁺ cells were enumerated from the lympho-monocyte fraction and cell numbers are represented as number of cells per million cytometric events.

Statistical Analysis

Cell numbers are expressed as mean \pm s.e.m per 10⁶ events. Normal distribution of the data was confirmed through Kolmogorov-Smirnov test. Skewed distributions were log transformed for statistical analysis. Comparisons between two groups and within groups were performed by the unpaired and paired Student's *t* test respectively for continuous variables. For non-parametric variables, Mann-Whitney's test and Wilcoxon matched pair test were employed. Correlation between cell counts and biochemical parameters were assessed with Spearman's Rank coefficient (ρ). Statistical significance was accepted if the null hypothesis could be rejected at $p < 0.05$. All analyses were done using Windows - based SPSS statistical software (version15.0).

Results

Characteristics of the study population

The morphometric, clinical and metabolic features of the study group are summarized in Table 1. None of the subjects were on medication at the time of recruitment and all provided written consent for the study. The pre-diabetes group had significantly higher body weight ($p = 0.026$), higher BMI ($p = 0.005$) and increased waist circumference ($p = 0.032$). The WHR was also higher for the pre-diabetes group though the increase did not reach level of significance. Among the glucose and insulin profiles, the pre-diabetes group had higher levels of fasting glucose, fasting insulin, post load glucose and post load insulin. Also the pre-diabetes group had greater insulin resistance as measured by HOMA-IR ($p = 0.0001$). The HbA_{1c} levels were significantly higher in pre-diabetes group compared to the NGT group. There was a marginal increase in the neutrophil counts which was approaching significance while the lymphocytes were decreased in the pre-diabetes group (Table 2 $p = 0.063$). For the benefit of the readers the clinical profiles of IFG and IGT subjects within the pre-diabetes group are further listed in Table 3. As seen in Table 3 the weight of IFG subjects was significantly higher than NGT. Both the IFG and IGT subjects were

Table 1 Anthropometric, clinical and biochemical characteristics of study subjects

Parameters	NGT (n = 24)	Pre-diabetes (n = 26)	p value
Anthropometric measures			
Age (years)	36 \pm 2	39 \pm 2	0.232
Weight (Kg)	69 \pm 2	77 \pm 3*	0.026
Body Mass Index (kg/m ²)	24.0 \pm 0.6	26.5 \pm 0.6*	0.005
Waist Circumference (cm)	90.3 \pm 1.7	98.3 \pm 3.1*	0.032
Waist - Hip Ratio	0.96 \pm 0.01	0.98 \pm 0.01	0.070
Systolic Blood Pressure (mmHg)	119.0 \pm 1.8	122.8 \pm 2.6	0.240
Diastolic Blood Pressure (mmHg)	76.3 \pm 1.6	76.6 \pm 1.9	0.992
Biochemical parameters			
OGTT			
Fasting plasma glucose (mmol/l)	5.0 \pm 0.1	5.7 \pm 0.1**	0.0001
2Hr Post plasma glucose (mmol/l)	5.6 \pm 0.2	8.1 \pm 0.3**	0.0001
Glycated Hemoglobin [HbA _{1c}] (%)	5.4 \pm 0.1	5.8 \pm 0.1*	0.0013
Fasting Insulin (pmol/l)	49.8 \pm 5	95.1 \pm 10**	0.0002
2Hr post Insulin (pmol/l)	325.8 \pm 49	780.0 \pm 75**	0.0001
HOMA-IR	1.9 \pm 0.2	4.0 \pm 0.4**	0.0001
Lipid profile			
Total Cholesterol (mmol/l)	4.6 \pm 0.2	4.9 \pm 0.2	0.376
Serum Triglycerides (mmol/l)	1.51 \pm 0.2	1.9 \pm 0.2	0.166
HDL Cholesterol (mmol/l)	1.0 \pm 0.04	0.9 \pm 0.04	0.459
LDL Cholesterol (mmol/l)	2.9 \pm 0.1	3.0 \pm 0.2	0.632
VLDL Cholesterol (mmol/l)	0.7 \pm 0.1	0.9 \pm 0.1	0.163
Others			
Serum Creatinine (μ mol/l)	79.6 \pm 1.8	106.9 \pm 27.1	0.364
Hs-CRP (mg/l)	2.5 \pm 0.5	3.1 \pm 0.5	0.341
Microalbuminuria (μ g/mg)	5.2 \pm 0.8	7.6 \pm 1.5	0.344

Values are expressed as mean \pm SEM. Hs - CRP indicates High sensitive C - reactive protein. Glycated Hemoglobin values are expressed in %.* $P < 0.05$ and ** $P < 0.001$.

Table 2 Hematology measurements of study subjects

Parameters	NGT (n = 24)	Pre-diabetes (n = 26)	p value
WBC count (10 ⁹ /l)	6.8 \pm 0.2	7.0 \pm 0.3	0.717
Neutrophils (%)	51.5 \pm 1.4	54.8 \pm 1.6	0.076
Lymphocytes (%)	35.5 \pm 1.3	32.3 \pm 1.1	0.063
Monocytes (%)	8.1 \pm 0.3	7.6 \pm 0.3	0.148
Eosinophils (%)	4.7 \pm 0.8	5.1 \pm 0.7	0.727
Basophils (%)	0.18 \pm 0.02	0.25 \pm 0.04	0.177
RBC count (10 ¹² /l)	5.1 \pm 0.1	5.2 \pm 0.1	0.750
Platelets (10 ⁹ /l)	243.5 \pm 7.7	247.8 \pm 10.7	0.747

Values are expressed as mean \pm SEM. Differential cell count values are expressed in %.

Table 3 Anthropometric, clinical and biochemical characteristics of study subjects

Parameters	NGT (n = 24)	IFG (n = 09)	IGT (n = 17)
Anthropometric measures			
Age (years)	36 ± 2	38 ± 2	40 ± 2
Weight (Kg)	69 ± 2	82 ± 4**	74 ± 3
Body Mass Index (kg/m ²)	24.0 ± 0.6	27.0 ± 1.3*	26.2 ± 0.7*
Waist Circumference (cm)	90.3 ± 1.7	97.0 ± 2.2*	99.0 ± 4.7
Waist - Hip Ratio	0.96 ± 0.01	0.97 ± 0.01	0.98 ± 0.01
Systolic Blood Pressure (mmHg)	119.0 ± 1.8	120.0 ± 1.9	124.3 ± 3.8
Diastolic Blood Pressure (mmHg)	76.3 ± 1.6	75.6 ± 1.9	77.1 ± 2.7
OGTT			
Fasting plasma glucose (mmol/l)	5.0 ± 0.1	5.7 ± 0.1***	5.7 ± 0.2***
2Hr Post plasma glucose (mmol/l)	5.6 ± 0.2	6.8 ± 0.3**	8.8 ± 0.2*** ##
Glycated Hemoglobin [HbA1c] (%)	5.4 ± 0.1	5.6 ± 0.1	5.9 ± 0.1***
Fasting Insulin (pmol/l)	49.8 ± 5	93.7 ± 19 **	95.8 ± 11***
2Hr post Insulin (pmol/l)	325.8 ± 49	653.1 ± 121**	1259 ± 231***
HOMA-IR	1.9 ± 0.2	3.9 ± 0.8***	4.0 ± 0.5***
Lipid profile			
Total Cholesterol (mmol/l)	4.6 ± 0.2	4.8 ± 0.4	4.9 ± 0.2
Serum Triglycerides (mmol/l)	1.51 ± 0.2	2.1 ± 0.4	1.8 ± 0.2
HDL Cholesterol (mmol/l)	1.0 ± 0.04	1.0 ± 0.09	1.0 ± 0.03
LDL Cholesterol (mmol/l)	2.9 ± 0.1	2.9 ± 0.3	3.1 ± 0.2
VLDL Cholesterol (mmol/l)	0.7 ± 0.1	0.9 ± 0.2	0.8 ± 0.1
Others			
Serum Creatinine (μmol/l)	76.6 ± 2.3	73.7 ± 2.6	124.5 ± 41.2#
Hs-CRP (mg/l)	2.5 ± 0.5	2.0 ± 0.2	3.7 ± 0.6
Microalbuminuria (μg/mg)	5.2 ± 0.8	4.2 ± 0.6	9.4 ± 2.1

Values are expressed as mean ± SEM. Hs - CRP indicates High sensitive C - reactive protein. Glycated Hemoglobin values are expressed in %. **P* < 0.05, ***P* < 0.001 & ****P* < 0.0001 vs NGT and # *P* < 0.05 & ## *P* < 0.0001 vs IFG.

insulin resistant as measured via HOMA-IR and their glucose and insulin profiles were accordingly higher than the NGT group.

Circulating progenitor cells in NGT versus Pre-diabetes group

Upon morphological gating of the lympho-monocyte fraction based on forward and side scatter, the CD34⁺, CD133⁺ and CD133⁺CD34⁺ cells were sorted following gating with appropriate fluorophore labeled corresponding isotype control antibodies (Figure 1). No differences were observed in the fasting levels of CD34⁺ cells between NGT and pre-diabetes subjects (1401 ± 417 vs

1751 ± 483, *p* = 0.589) or for CD133⁺ cells (34 ± 12 vs 52 ± 22, *p* = 0.874). Here the cell numbers are depicted for every one million cytometric events counted. Similarly no differences were seen in the circulation for fasting levels of CD133⁺CD34⁺ cells between the two groups (347 ± 114 vs 295 ± 69, *p* = 0.392 for NGT versus pre-diabetes respectively).

However at 2 hour post glucose load there was almost a 3-fold increase in the CD34⁺ cell count in the NGT group (*p* = 0.003) which appeared to be attenuated in the pre-diabetes group with the *p* value approaching significance (refer to Figure 2A, *p* = 0.066). No increase in the CD133⁺ cells following glucose load was seen in either of the study groups (data not shown). Similarly a 2.5-fold increase was seen for CD133⁺CD34⁺ cells in the NGT group (*p* = 0.019) at 2 hours following 75 g glucose load, which was blocked in pre-diabetes group (Figure 2B, *p* = 0.024).

Furthermore when the pre-diabetes group was further split into IFG and IGT, the glucose mediated increase in CD34⁺ and CD133⁺CD34⁺ was significantly blocked for the IGT subjects (Figure 3). Intriguingly for the IFG group there appeared to be an increase in the fasting levels of CD34⁺ cells compared to NGT (Figure 3A) but the *p* value did not reach significance (*p* = 0.133). With regard to CD133⁺CD34⁺ cells the fasting levels of these progenitor cells did not vary among the group though the glucose-load induced increase appeared blunted for IFG (Figure 3B). It should however be noted that these observations are made with limited number of volunteers subjected to a restricted OGTT analysis which scores cell numbers only at fasting and 2 hour post load stages. This hence warrants for a detailed analysis of progenitor cell kinetics every 30 minutes following glucose challenge in future studies.

Correlation Analysis

Spearman's Rank correlation analysis was performed to determine correlation between progenitor cell numbers and biochemical parameters. A positive correlation was observed between CD34⁺ and CD133⁺CD34⁺ cells both during fasting and 2 hour post glucose load. The Rho value for fasting cell counts was 0.270 (*p* = 0.058) while for 2 hour values the Rho between the two cell types was 0.361 (*p* = 0.01). When fasting cell counts for CD34⁺ were analyzed, no appreciable correlation was seen with fasting profiles of glucose or insulin, however fasting CD34⁺ cells showed a near significant inverse correlation with cholesterol (*ρ* = -0.263, *p* = 0.064), LDL (*ρ* = -0.259, *p* = 0.070) and serum creatinine (*ρ* = -0.249, *p* = 0.084). The fasting cell counts for CD133⁺CD34⁺ cells exhibit a near significant and significant positive correlation with fasting glucose (*ρ* = 0.245, *p* = 0.086) and post load glucose (*ρ* = 0.297, *p* = 0.036) respectively. Serum

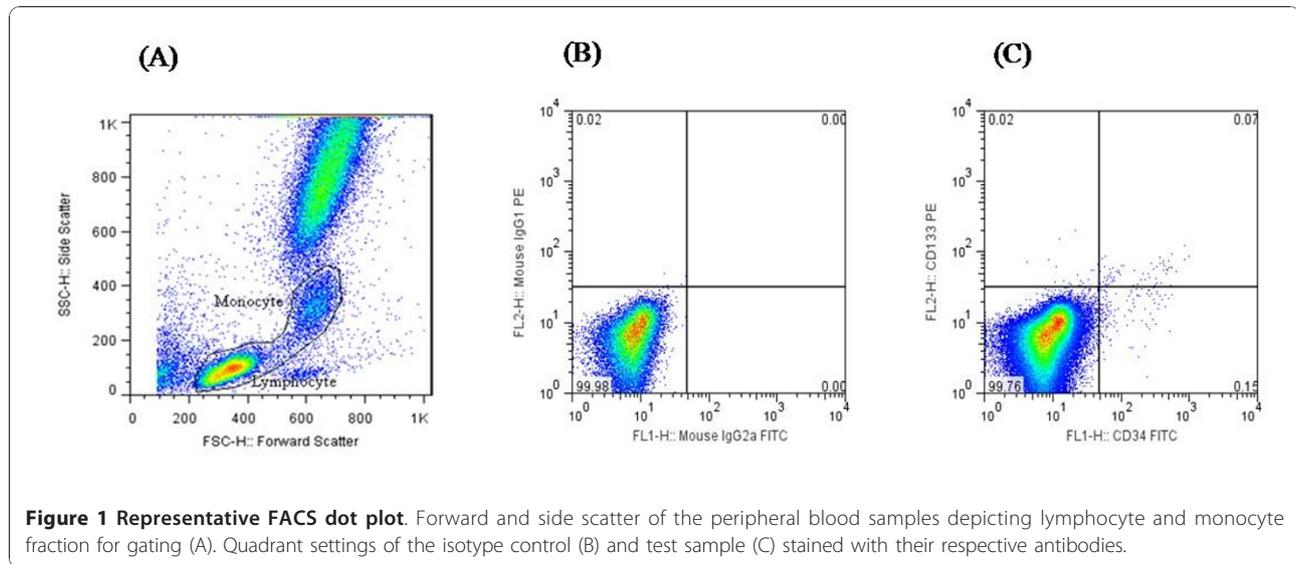


Figure 1 Representative FACS dot plot. Forward and side scatter of the peripheral blood samples depicting lymphocyte and monocyte fraction for gating (A). Quadrant settings of the isotype control (B) and test sample (C) stained with their respective antibodies.

creatinine levels exhibited inverse correlation with post glucose load cell counts of CD133⁺CD34⁺ cells ($\rho = -0.312$, $p = 0.023$). Intriguingly the CD34⁺ cell counts following glucose load demonstrated inverse correlation with neutrophils, with $\rho = -0.330$ ($p = 0.019$).

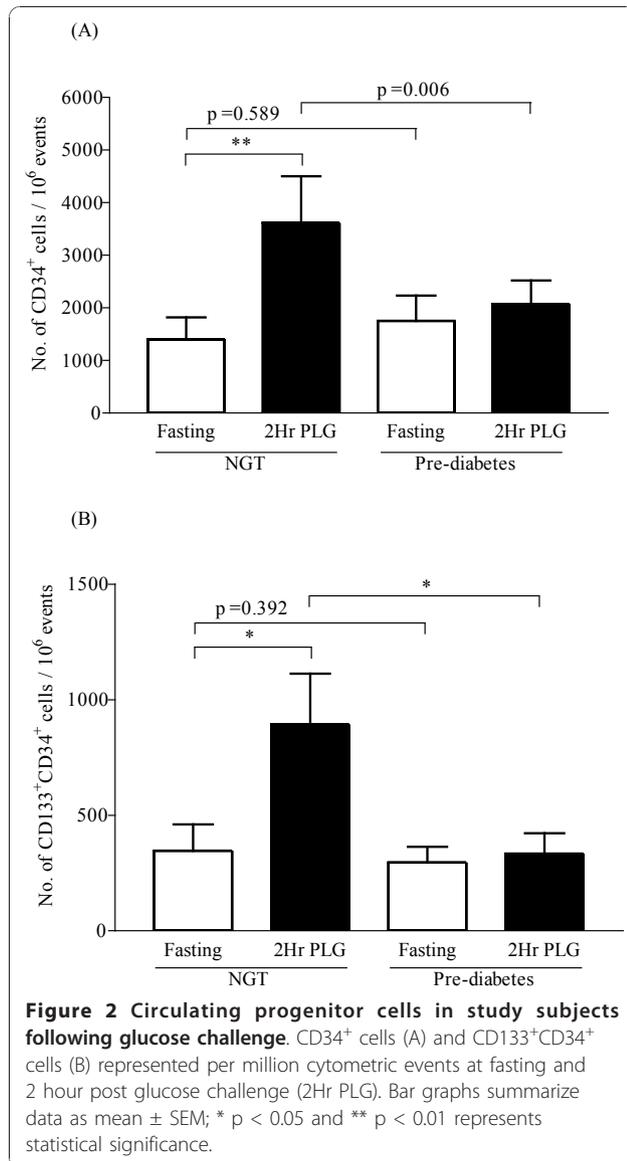
Discussion

In the current study, we report a 2 to 3-fold increase in the circulating levels of CD34⁺ and CD133⁺CD34⁺ progenitor cells in healthy Asian Indian males in response to 75 g glucose load. This increase in progenitor cell counts is however attenuated in pre-diabetes subjects particularly IGT. To the best of our knowledge this is the first study to identify an increase in circulating progenitor cells in response to glucose load in healthy individuals and its attenuation in IGT. A positive correlation was seen between CD34⁺ and CD133⁺CD34⁺ cells both at fasting and post load stages, confirming a previous finding that double positive cells are a subset of the CD34⁺ pool [19]. As previously reported we also observed an inverse correlation of fasting CD34⁺ cell counts with cholesterol and LDL [19], however the p values for these correlations did not reach significance possibly due to small sample size. Intriguingly the CD34⁺ cell counts following glucose load showed inverse correlation with neutrophils ($p = 0.019$). Additionally the post load CD133⁺CD34⁺ counts were inversely correlated with serum creatinine.

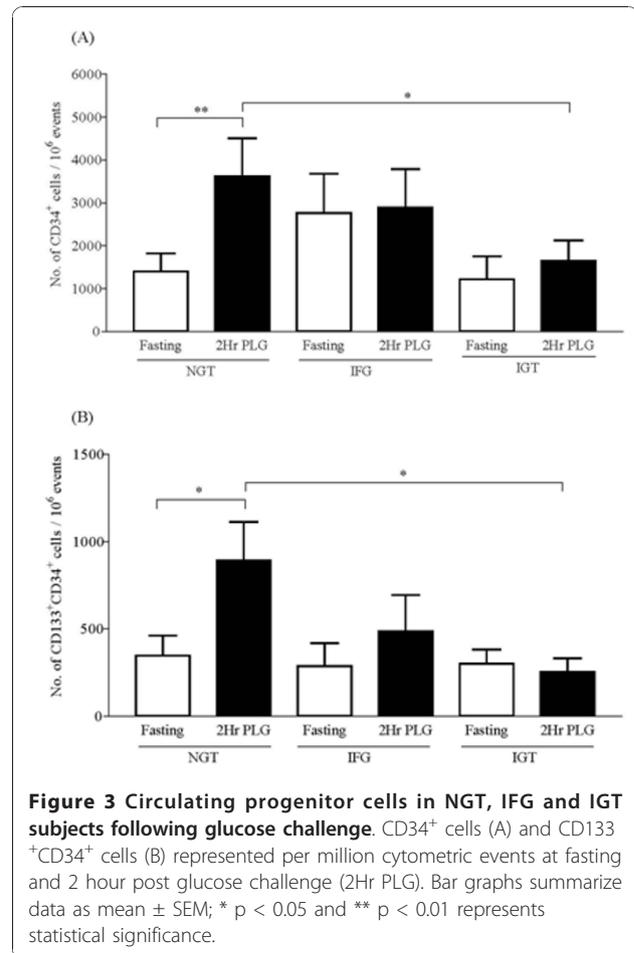
Progenitor cells besides giving rise to EPCs can integrate into skeletal muscle, neurons or myo-endothelial cells [1]. They exhibit better dispersion in host muscle tissue following cryo injury and can form capillary like structures [20]. Similarly CD133⁺CD34⁺ cells exhibit a strong correlation with degree of engraftment in multiple myeloma patients undergoing autologous

transplantation [21]. It is of interest that a recent study also claims that HSC transplantation imparts insulin independency in early type I diabetes patients [22]. Although the knowledge about mobilization of various fractions of haematopoietic stem cells and the endogenous processes governing physiological organ recoveries mediated by them are scarce, it is tempting to speculate that increases in circulating progenitor cell counts in response to glucose load is possibly the body's precautionary response to protect various tissues and organs from glycemic insults. In the likelihood of this possibility the inverse correlation of CD133⁺CD34⁺ cells ($\rho = -0.312$, $p = 0.023$) with serum creatinine is indeed interesting. However these findings need to be tested and characterized in experimental models of diabetes.

Unlike a recently published study by Fadini et al. [14], we did not observe any variations in the fasting cell counts of CD34⁺ progenitor cells between the two groups possibly due to small sample size. We however observed an attenuated response at 2 hours post glucose challenge for CD34⁺ and CD133⁺CD34⁺ cells in the pre-diabetes group compared to the NGT group. Although the mechanisms behind this blunted response and the functional consequences of this aberration are presently unknown it can be envisaged that hostile vascular environment in pre-diabetes either due to reactive oxygen species or inflammation may affect the mobilization of progenitor cells from bone marrow. It should be noted that pre-diabetes is associated with increased oxidative stress and circulatory pro-inflammatory cytokines [15,23,24]. Given that the post glucose load-cell counts for CD34⁺ inversely correlated with neutrophil counts, it is likely that decrease in progenitor count is an effect of increased inflammatory



state. Since we did not assess the oxidative stress in these subjects, it is difficult at this stage to comment on the influence of reactive oxygen species on mobilization of progenitors from bone marrow in pre-diabetes subjects. Alternatively it is likely that reduction in post load progenitor cell counts in pre-diabetes subjects is due to accelerated progenitor cell senescence which unfortunately was not investigated in this study. Ageing is known to restrict the bone marrow reserve of progenitor cells and reduce circulating numbers of these cells [25,26]. Hence this study was performed with age matched younger subjects. The other advantage of this study is the recruitment of drug naïve NGT and pre-diabetes subjects. Thus any compounding effects of either medication or age are minimized and the study provides a reasonable indication of



influence of glucose challenge on progenitor cell count. It is likely that blunted post load response of progenitor cells may precede the actual decrease seen under fasting conditions for these cells during the course of developing diabetes [7,10], thereby suggesting that insults to vasculature and possibly other organs occur prior to the onset of overt diabetes diagnosed through fasting glucose levels [27]. Finally whether similar progenitor dynamics with regard to post glucose challenge is seen in other populations prior to or during the onset of diabetes is currently unknown and this points to need for replication of the findings in other populations.

Limitations

There are certain limitations of the current study. For example due to small sample size certain observations did not reach levels of statistical significance. Similarly although in the IFG subjects there appears to be an increase in the fasting levels of CD34⁺ cells as opposed to NGT, it fails to reach statistical significance. Additionally due to small sample size we could not perform sub-group

analysis and being observational in nature, the causative factors could not be determined. The other limitation of the study is a restricted OGTT analysis with measurement of glucose and insulin only at fasting and 2 hour post glucose challenge. A detailed analysis every 30 minutes would give a better perspective on progenitor cell kinetics as a function of metabolic parameters. Finally the study addresses the progenitor cell dynamics in Asian Indian male subjects, whether similar dynamics are seen in female subjects' remains to be seen.

Conclusions

Glucose challenge increases circulating levels of CD34⁺ and CD133⁺CD34⁺ haematopoietic stem cells in healthy subjects which is compromised in subjects with IGT.

Abbreviations

OGTT: Oral Glucose Tolerance Test; IFG: Impaired fasting glucose; IGT: Impaired glucose tolerance; NGT: Normal glucose tolerance; HOMA-1R: Homeostasis model assessment of insulin resistance; EPC: Endothelial progenitor cell; HSC: Haematopoietic stem cell; CAD: Coronary artery disease; CVD: Cardiovascular disease; FACS: Fluorescence assisted cell sorting; BMI: Body mass index; WHR: Waist to hip ratio.

Acknowledgements

This work was supported by the Innovative Young Biotechnologist Award (IYBA) program of the Department of Biotechnology (DBT), Government of India awarded to Dr. Madhulika Dixit. Authors acknowledge Mrs. N. Alamelu, Proprietress, ANSI Techniques, Chennai, for extending her help in statistical analysis. Help rendered by Dr. V. Kumaraswamy, Director, Tuberculosis Research Center (TRC), Dr. M. Deepa and Dr. H. Ranjani of Dr. Mohan's Diabetes Specialties Centre and Madras Diabetes Research Foundation, Gopalapuram, Chennai is acknowledged.

Author details

¹Laboratory of Vascular Biology, Department of Biotechnology, Indian Institute of Technology Madras (IIT Madras), Chennai, India. ²Department of Diabetology, Madras Diabetes Research Foundation (MDRF) & Dr. Mohan's Diabetes Specialties Centre: WHO Collaborating Centre for Non-Communicable Diseases Prevention and Control and IDF Centre for Education, Gopalapuram, Chennai, India. ³National Institutes of Health-International Center for Excellence in Research, Chennai, India and SAIC Frederick, Inc., NCI Frederick, Frederick, Maryland, USA.

Authors' contributions

AAN executed the study and analyzed data, VM screened patients and reviewed manuscript, SSB analyzed data and reviewed manuscript, SB analyzed data and MD planned the study, reviewed the data and wrote the manuscript. All authors have read and approved the final manuscript.

Competing interests

The authors declare that they have no competing interests.

Received: 12 November 2010 Accepted: 11 January 2011
Published: 11 January 2011

References

1. Krause DS, Theise ND, Collector MI, Henegariu O, Hwang S, Gardner R, Neutzel S, Sharkis SJ: **Multi-organ, multi-lineage engraftment by a single bone marrow-derived stem cell.** *Cell* 2001, **105**:369-377.
2. Yeh ET, Zhang S, Wu HD, Korbli M, Willerson JT, Estrov Z: **Transdifferentiation of human peripheral blood CD34⁺-enriched cell population into cardiomyocytes, endothelial cells, and smooth muscle cells in vivo.** *Circulation* 2003, **108**:2070-2073.
3. Abrahamsen JF, Smaaland R, Sothorn RB, Laerum OD: **Variation in cell yield and proliferative activity of positive selected human CD34⁺ bone marrow cells along the circadian time scale.** *Eur J Haematol* 1998, **60**:7-15.
4. Grundmann F, Scheid C, Braun D, Zobel C, Reuter H, Schwinger RH, Muller-Ehmsen J: **Differential increase of CD34, KDR/CD34, CD133/CD34 and CD117/CD34 positive cells in peripheral blood of patients with acute myocardial infarction.** *Clin Res Cardiol* 2007, **96**:621-627.
5. Fadini GP, de KS, Albiero M, Coracina A, Pagnin E, Baesso I, Cignarella A, Bolego C, Plebani M, Nardelli GB, Sartore S, Agostini C, Avogaro A: **Gender differences in endothelial progenitor cells and cardiovascular risk profile: the role of female estrogens.** *Arterioscler Thromb Vasc Biol* 2008, **28**:997-1004.
6. Almdal T, Scharling H, Jensen JS, Vestergaard H: **The independent effect of type 2 diabetes mellitus on ischemic heart disease, stroke, and death: a population-based study of 13,000 men and women with 20 years of follow-up.** *Arch Intern Med* 2004, **164**:1422-1426.
7. Fadini GP, Sartore S, Albiero M, Baesso I, Murphy E, Menegolo M, Grego F, Vigili de KS, Tiengo A, Agostini C, Avogaro A: **Number and function of endothelial progenitor cells as a marker of severity for diabetic vasculopathy.** *Arterioscler Thromb Vasc Biol* 2006, **26**:2140-2146.
8. Fadini GP, Agostini C, Sartore S, Avogaro A: **Endothelial progenitor cells in the natural history of atherosclerosis.** *Atherosclerosis* 2007, **194**:46-54.
9. Fadini GP, Boscaro E, de KS, Agostini C, Seeger F, Dimmeler S, Zeiher A, Tiengo A, Avogaro A: **Time course and mechanisms of circulating progenitor cell reduction in the natural history of type 2 diabetes.** *Diabetes Care* 2010, **33**:1097-1102.
10. Jung C, Rafnsson A, Shemyakin A, Bohm F, Pernow J: **Different subpopulations of endothelial progenitor cells and circulating apoptotic progenitor cells in patients with vascular disease and diabetes.** *Int J Cardiol* 2010, **143**:368-372.
11. Murphy C, Kaganayagam GS, Jiang B, Chowienczyk PJ, Zbinden R, Saha M, Rahman S, Shah AM, Marber MS, Kearney MT: **Vascular dysfunction and reduced circulating endothelial progenitor cells in young healthy UK South Asian men.** *Arterioscler Thromb Vasc Biol* 2007, **27**:936-942.
12. Dessapt C, Karalliedde J, Hernandez-Fuentes M, Martin PP, Maltese G, Dattani N, Atkar R, Viberti G, Gnudi L: **Circulating vascular progenitor cells in patients with type 1 diabetes and microalbuminuria.** *Diabetes Care* 2010, **33**:875-877.
13. Fadini GP, Miorin M, Facco M, Bonamico S, Baesso I, Grego F, Menegolo M, de Kreutzenberg SV, Tiengo A, Agostini C, Avogaro A: **Circulating endothelial progenitor cells are reduced in peripheral vascular complications of type 2 diabetes mellitus.** *J Am Coll Cardiol* 2005, **45**:1449-1457.
14. Fadini GP, Pucci L, Vanacore R, Baesso I, Penno G, Balbarini A, Di SR, Miccoli R, de KS, Coracina A, Tiengo A, Agostini C, Del PS, Avogaro A: **Glucose tolerance is negatively associated with circulating progenitor cell levels.** *Diabetologia* 2007, **50**:2156-2163.
15. Bonora E: **Postprandial peaks as a risk factor for cardiovascular disease: epidemiological perspectives.** *Int J Clin Pract Suppl* 2002, 5-11.
16. Meigs JB, Nathan DM, D'Agostino RB, Wilson PW: **Fasting and postchallenge glycemia and cardiovascular disease risk: the Framingham Offspring Study.** *Diabetes Care* 2002, **25**:1845-1850.
17. **Glucose tolerance and mortality: comparison of WHO and American Diabetes Association diagnostic criteria. The DECODE study group. European Diabetes Epidemiology Group. Diabetes Epidemiology: Collaborative analysis Of Diagnostic criteria in Europe.** *Lancet* 1999, **354**:617-621.
18. Distler JH, Allanore Y, Avouac J, Giacomelli R, Guiducci S, Moritz F, Akhmetshina A, Walker UA, Gabrielli A, Muller-Ladner U, Tyndall A, Matucci-Cerinic M, Distler O: **EULAR Scleroderma Trials and Research group statement and recommendations on endothelial precursor cells.** *Ann Rheum Dis* 2009, **68**:163-168.
19. Fadini GP, de Kreutzenberg SV, Coracina A, Baesso I, Agostini C, Tiengo A, Avogaro A: **Circulating CD34⁺ cells, metabolic syndrome, and cardiovascular risk.** *Eur Heart J* 2006, **27**:2247-2255.
20. Burt RK, Loh Y, Pearce W, Beohar N, Barr WG, Craig R, Wen Y, Rapp JA, Kessler J: **Clinical applications of blood-derived and marrow-derived stem cells for nonmalignant diseases.** *JAMA* 2008, **299**:925-936.
21. Hicks C, Wong R, Manoharan A, Kwan YL: **Viable CD34⁺/CD133⁺ blood progenitor cell dose as a predictor of haematopoietic engraftment in**

- multiple myeloma patients undergoing autologous peripheral blood stem cell transplantation. *Ann Hematol* 2007, **86**:591-598.
22. Voltarelli JC, Couri CE: **Stem cell transplantation for type 1 diabetes mellitus.** *Diabetol Metab Syndr* 2009, **1**:4.
 23. Deepa R, Velmurugan K, Arvind K, Sivaram P, Sientay C, Uday S, Mohan V: **Serum levels of interleukin 6, C-reactive protein, vascular cell adhesion molecule 1, and monocyte chemoattractant protein 1 in relation to insulin resistance and glucose intolerance—the Chennai Urban Rural Epidemiology Study (CURES).** *Metabolism* 2006, **55**:1232-1238.
 24. Anderson RA, Evans ML, Ellis GR, Graham J, Morris K, Jackson SK, Lewis MJ, Rees A, Frenneaux MP: **The relationships between post-prandial lipaemia, endothelial function and oxidative stress in healthy individuals and patients with type 2 diabetes.** *Atherosclerosis* 2001, **154**:475-483.
 25. Heiss C, Keymel S, Niesler U, Ziemann J, Kelm M, Kalka C: **Impaired progenitor cell activity in age-related endothelial dysfunction.** *J Am Coll Cardiol* 2005, **45**:1441-1448.
 26. Keymel S, Kalka C, Rassaf T, Yeghiazarians Y, Kelm M, Heiss C: **Impaired endothelial progenitor cell function predicts age-dependent carotid intimal thickening.** *Basic Res Cardiol* 2008, **103**:582-586.
 27. Hu FB, Stampfer MJ, Haffner SM, Solomon CG, Willett WC, Manson JE: **Elevated risk of cardiovascular disease prior to clinical diagnosis of type 2 diabetes.** *Diabetes Care* 2002, **25**:1129-1134.

Pre-publication history

The pre-publication history for this paper can be accessed here:
<http://www.biomedcentral.com/1472-6823/11/2/prepub>

doi:10.1186/1472-6823-11-2

Cite this article as: Nathan *et al.*: Glucose challenge increases circulating progenitor cells in Asian Indian male subjects with normal glucose tolerance which is compromised in subjects with pre-diabetes: A pilot study. *BMC Endocrine Disorders* 2011 **11**:2.

**Submit your next manuscript to BioMed Central
and take full advantage of:**

- Convenient online submission
- Thorough peer review
- No space constraints or color figure charges
- Immediate publication on acceptance
- Inclusion in PubMed, CAS, Scopus and Google Scholar
- Research which is freely available for redistribution

Submit your manuscript at
www.biomedcentral.com/submit

