Author's response to reviews

Title: 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.

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Author's response to reviews: see over
Reviewer: Franco Folli

1. Comment 1: Why did you group the subjects in the Prediabetes category? I would keep IFG and IGT as separate groups.

Answer: Both IFG and IGT subjects are at high risk of developing diabetes and based on ADA guidelines [1] are considered to be pre-diabetic since their fasting glucose profiles though do not meet the diagnostic mark are still way above the normal values. Since both the categories of subjects are insulin resistant and given that both the groups are associated with increased cardiovascular risks [2] we chose to group them together as pre-diabetes. However we do recognize that the sites of insulin resistance in these subjects differ with IFG having hepatic insulin resistance and IGT exhibiting moderate to severe muscle insulin resistance [3,4]. We have thus taken the reviewer’s suggestion and have additionally represented the data as NGT, IFG and IGT in figure 3 with accompanying clinical data in table 3.

2. Comment 2: What is the correlation between the various metabolic parameters and the number of CD34 and CD133CD34 positive cells in the whole population (NGT+IFG+IGT). I think a multivariate analysis would be most appropriate to evaluate the major metabolic determinants of the described alterations. I think that this is continuous phenomenon and it should be described as such.

Answer: We wish to bring to reviewer’s notice that cell counts for CD34+ cells in the whole population did not exhibit a continuous phenomenon and followed a non-parametric distribution and despite transformations the data was skewed and thus we could not perform routine linear regression and multivariate analysis. Driven by this constraint we thus performed a Spearman’s Rank correlation which is mentioned in the results section of the manuscript. As mentioned in the ‘Correlation Analysis’ section on page 10, we did observe a near significant inverse correlation between CD34+ cells with cholesterol (ρ=-0.263, p=0.064), LDL (ρ=-0.259, p=0.070) and serum creatinine (ρ=-0.249, p=0.084). CD133-CD34+ cells on the other hand showed a positive correlation with glucose values. All these correlations however failed to reach statistical significance possibly due to small sample size and this has been mentioned in the discussion as the limitation of the study.

3. Comment 3: The group of Dr. Fadini has published extensively on this topic and I would like to know what is the difference between this work and the work of Dr. Fadini?

Answer: This is indeed an important question given that Fadini et.al. have pioneered the study of circulating endothelial progenitor cells in patients exhibiting diabetes and metabolic syndrome [5,6]. In two of their recent studies Fadini et.al. documented a decrease in circulating levels of CD34+ cells in IGT subjects at fasting stage [7,8]. We however fail to detect any decline in the circulating levels of progenitor cells at fasting stage in IGT subjects in the current study possibly due to lesser sample size. It should be however noted that in their study the subjects were at-least 10 years older than the subjects recruited in this study and it is well known that ageing reduces circulating levels of progenitor cells. Secondly Fadini et.al. did not study the effect of glucose challenge on
dynamics of progenitor cells in circulation while the current study reports for the first time an increase in circulating levels of CD34+ and CD133+CD34+ progenitor cells following 75g glucose load in healthy subjects (i.e. NGT). This effect of glucose load was however found to be lost in subjects with insulin resistance (i.e. IFG and IGT).

Reference List

Reviewer 2: Giovanni Pacini

1. Comment 1: The only major point from this reviewer is the low number of subjects to obtain conclusive evidences. The authors are well aware of this and cite this aspect a few times in the Discussion. My recommendation is a short paragraph titled “limitations” where this and other aspects are clearly stated.

Answer: We thank the reviewer for this suggestion and have accordingly included a section on “limitations” in the revised manuscript.

2. Unfortunately the OGTT was very limited: only glucose at fasting and 2h and insulin---I
believe---only at fasting. HOMA is the only method possible in these circumstances, for evaluation of insulin resistance. A better OGTT would have given several other parameters which might show relationships with progenitor changes.

Answer: We thank the reviewer for pointing out this aspect of the study and we do agree with him that the OGTT performed was indeed limited as we measured glucose and insulin both at fasting and 2 hour only and did not perform a detailed analysis every 30 minutes. We have now included this point in the “Limitations” section of the revised manuscript. This kind of OGTT was essentially performed as a diagnostic test in this pilot study as per the recommendation of the institutional ethics committee in order to minimize distress to the volunteers. However now having some clue on the kinetics of progenitor cells in response to glucose load, we plan to carry out detailed OGTT for our future studies.

3. The difference in HOMA could be due to different body weight. The authors were cautious about age; is there any evidence on the possible role played weight?

Answer: In literature numerous studies have shown correlation of HOMA with adiposity, BMI and body composition [1-3]. Based on reviewer’s suggestion we carried out a Pearson’s correlation analysis between weight and HOMA for this study population and found a strong correlation of 0.518 with a p value of 0.0002 between the two.

4. The aim of the study MUST be clearly reported in the Abstract.
Answer: As recommended by the reviewer we have added a statement to this effect in the Abstract.

Reference List