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Dear Editor,

Please find enclosed the edited manuscript in Word format

Title: CD36 expression and lipid metabolism post oral glucose challenge in South Asians

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The manuscript has been improved according to the suggestions of reviewers:

1 Format has been updated and English revised.

2 comments from reviewers (responses in bold)

1. Abstract “while cholesterol (but not triglyceride) concentrations within very low density lipoprotein (VLDL) and low density lipoprotein (LDL) subfractions increased ($P<0.001$).” These phrases are not right because cholesterol concentrations within very low density lipoprotein (VLDL) showed $P=0.001$ and low density lipoprotein (LDL) subfractions showed $P=0.003$.

Thank you for the correction. The abstract has been changed to quote the P value as $P<0.01$.

2. Table2. All data are presented as median and interquartile. Does it mean that all data in Table 2 are distributed non-parametrically?

Other than the serum cholesterol levels and plasma glucose levels, we can confirm that all measures in table 2 are distributed non-parametrically (K-S plots). We chose to present all data as median and interquartile ranges in an attempt to standardize the data for the reader. The median and mean levels for glucose and cholesterol bear close resemblance.

3. Table2. The difference between LDL cholesterol (2.03 mmol/L) and LDL subfraction cholesterol (1.30 mmol/L) is too much although the reviewer knows authors used the different methods to determine them.

Thank you for highlighting this discrepancy. LDL cholesterol was measured directly from a serum sample, whereas the LDL subfraction cholesterol was measured from plasma that had reconstituted with saline and KBr solution, ultra-centrifuged and extracted by hand. During each extraction, there were anticipated losses in cholesterol as lipid supernatant was aspirated from a polypropylene tube using a pump. As a result, direct measures of LDL cholesterol are higher than the subfraction concentrations of LDL cholesterol and are not directly comparable. Up to a 40% loss in the recovery of lipids during ultracentrifugation is common and reported elsewhere (Winocour PH et al. *Atherosclerosis*. 1991;89:49-57). The method used to separate lipoprotein subfractions in our study was the same as that used in the Collaborative Atorvastatin Diabetes Study (CARDS) as described by Mackness & Durrington (*Lipoprotein separation and analysis for clinical studies*. in: C.A. Converse, E.R. Skinner (Eds.) *Lipoprotein analysis. a practical approach*. Oxford University Press, Oxford; 1992:1–42.).

We have made a comment regarding this limitation in the discussion section.

4. Table2. Similarly, serum cholesterol (3.41 mmol/L) should be expected the same value of total cholesterol from HDL (1.27 mmol/L), LDL (2.03 mmol/L), VLDL (1.26 mmol/L), and others. Serum cholesterol is much less than gathered cholesterol (4.56 mmol/L) from HDL, LDL, and VLDL.

We would like to kindly explain that serum cholesterol (total cholesterol) is that concentration of cholesterol that is not associated with HDL. As such, the serum cholesterol should reflect those concentrations recovered from LDL, IDL (intermediate density lipoprotein) and VLDL. In the data quoted above, the serum cholesterol 3.41 mmol/l using direct analysis of plasma reflects the sum from ultracentrifuge separated VLDL (1.26 mmol/l) and LDL (2.03 mmol/l). Discrepancies are likely to result from losses during subfraction extraction and because estimations of denser LDL particles and IDL were not recovered.

Please see the comments above also.

5. Table4. Please describe which method is used for calculation of the P value between ANOVA and Friedman test.

The Friedman test was used for these measures.

6. Table4. It is very strange that serum cholesterol did not change although LDL cholesterol and VLDL cholesterol increased greatly and HDL2 cholesterol and HDL3 cholesterol decreased very modestly at 120 min. 7. The last paragraph: "In summary, these data describe changes in lipid metabolism following the oral ingestion of glucose in South Asians which includes the generation of VLDL, and an increase in monocytes expressing CD36." Please explain how it is different from other races without diabetes in lipid metabolism after a 75g oral glucose challenge.

Changes in levels of serum cholesterol (or total cholesterol) are quite robust and are established not reflect acute changes in fasting status or diet (which is not the case for non-esterified fatty acids and triglycerides). Within our lipid clinic, changes in serum cholesterol following statin therapy are not anticipated until at least 2 weeks of lipid lowering therapy. We propose that a lack of change in serum total cholesterol is due to this. As for the changes in VLDL and LDL cholesterol concentrations, one is tempted to speculate that the determination of cholesterol concentrations by an ultracentrifuge approach is sensitive to acute changes in metabolism by virtue of relative changes

in lipoprotein density. For example, following the oral glucose load, the concentrations of lipoprotein particles with a very low density (this may include chylomicrons and associated remnants) increased at the expense of intermediate density lipoproteins (IDL). Determinations of serum total cholesterol would not reflect this as the total cholesterol includes VLDL, LDL, IDL as a composite. Logistically, it would have been impossible to have accurately measured levels of small dense LDL and IDL (i.e. those particles with a density between 1.063 and 1.125 kg/l) in this study given the losses at each stage of ultracentrifugation. We were able to separate lipoproteins at a density ≤ 1.006 kg/l (i.e. VLDL), at 1.063 kg/l (i.e. LDL) and those above 1.125 kg/l in this study (i.e. HDL subclasses). Further description has been added to the methods section.

Abnormal increases in non-esterified fatty acid concentrations following the ingestion of a 75g oral glucose load have been demonstrated in South Asians by Kooner et al (ATVB 1998;18(7):1021-6). Kooner and co-workers reported that such changes were not evident amongst matched controls from the general White population (non diabetic). We propose that changes in NEFA impact on the relative densities of cholesterol carrying particles but we are unable to find such data in the literature.

3 References and typesetting were corrected and a comment was added.

We would like to thank the Editor and the reviewers for their time and support with this manuscript and we hope that the manuscript is sufficiently improved for publication.

Yours Sincerely

Jeetesh V Patel.