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***Observational Study***

**CD36 expression and lipid metabolism following an oral glucose challenge in South Asians**

Patel JV *et al.* CD36 and lipoprotein changes in South Asians

Jeetesh V Patel, Amitava Banerjee, Silvia Montoro-Garcia, Eduard Shantsila, Mushfique Alam, Paul Flinders, Kathleen AL Houlton, Elizabeth A Hughes, Gregory YH Lip, Paramjit S Gill

**Jeetesh V Patel, Amitava Banerjee, Silvia Montoro-Garcia, Eduard Shantsila, Elizabeth A Hughes, Gregory YH Lip,** University of Birmingham Centre for Cardiovascular Sciences, Sandwell and West Birmingham Hospitals NHS Trust, B71 4HJ West Midlands, United Kingdom

**Mushfique Alam, Paramjit S Gill,** Primary Care Clinical Sciences, University of Birmingham, B15 2TT West Midlands, United Kingdom

**Paul Flinders, Kathleen AL Houlton,** Medical School, University of Nottingham, B15 2TT West Midlands, United Kingdom

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**Correspondence to: Dr. Jeetesh V Patel,** Sandwell Medical Research Unit,Sandwell General Hospital, Lyndon, B71 4HJ West Bromwich, United Kingdom. jeeteshp@gmail.com

**Telephone:** +44-121-5073971

**Fax:** +44-121-5073216

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**Abstract**

**AIM:** To investigate lipid metabolism and the relationship with monocyte expression of the fatty acid translocase CD36 in South Asians.

**METHODS:** An observational study of South Asians whom as an ethnic group have- a higher risk of developing diabetes. The susceptibility to diabetes is coupled with an earlier and more rapid progression of micro-, and macro-vascular complications. Twenty-nine healthy South Asian participants [mean age 34.6 (8.9) years, 76.2% male, mean body-mass index 25.0 (5.2) kg/m2] were recruited from an urban residential area of central Birmingham (United Kingdom). The main outcomes measured were post prandial (30 min) and post absorptive (120 min) changes from fasting (0 min) in circulating lipoproteins, lipds and hormones, and monocyte expression of CD36 post injestion of a 75 g oral glucose challenge. The inducements of variations of monocyte CD36 expression were analysed.

**RESULTS:** Our results showed evident changes in monocyte CD36 expression following the glucose challenge (*P* < 0.001). Non-esterified fatty acids (NEFA) levels decreased progressively during the challenge (*P* < 0.001), in contrast to increased cholesterol (but not triglyceride) concentrations within very low density lipoprotein (VLDL) and low density lipoprotein subfractions (*P* < 0.01). Levels of, glucose, serum triglycerides and high density lipoprotein cholesterol remained largely unchanged. Variations of monocyte CD36 were negatively (r = -0.47, *P* = 0.04) associated to fat from the diet and positively to carbohydrate from the diet (r = 0.65, *P* < 0.001).

**CONCLUSION:** These data suggest that the initiation of VLDL genesis follows the consumption of glucose within this population, inferring that the sequestration of NEFA from these particles happens due to the increased availability of CD36 receptors. While these are preliminary results, it would appear that lifestyle exposures have a role in moderating the expression of CD36.

**Key words:** CD36; Lipoprotein; Glucose; South Asians; Diabetes; Micro-vascular; Macro-vascular

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**Core tip:** This study investigated the relationship between the expression of fatty acid translocase CD36 on monocytes and lipid and lipoprotein metabolism in South Asians. Post prandial and post absorptive changes from fasting in circulating lipids, lipoproteins, hormones and monocyte expression of CD36 were recorded subsequent to an oral glucose challenge. Our results showed discernible changes in monocyte CD36 expression post glucose administration. These data suggest that the production of very low density lipoprotein occurs subsequent to the ingestion of glucose within this population. It is presumed that the sequestration of non-esterified fatty acids from these particles happens due to an increase in availability of CD36 receptors.

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**INTRODUCTION**

The prevalence of diabetes is increased on the Indian subcontinent[1]. Globally dispersed migrant populations of ‘South Asians’ also have high rates of diabetes, which is evident when compared to the various indigenous populations of where they migrated[2-6]. Nephropathy and retinopathy develop earlier and are more progressive in South Asian diabetics compared to White diabetics[7-11]. The incidence of vascular disease is also higher in South Asians compared to the general White population[12].

Established risk factors such as obesity and urbanised lifestyle “operate” amongst South Asians but it remains unclear as to how far they explain an increased susceptibility to diabetes for this group. Rates of glucose intolerance amongst Indians living in rural village settings appear to be no different to their migrant contemporaries living overseas in Western countries[5,6]. We and others have observed that high glucose excursions in South Asians are underpinned by unregulated non-esterified fatty acids (NEFA) metabolism[13,14]. This phenomenon is likely to be complex and multifactorial, and we were interested to investigate the role of the fatty acid translocase CD36[15], which is the major facilitator of NEFA sequestration from the blood[16]. Specifically, CD36 allows the transport of NEFA that are generated from the lipolysis of triglyceride-rich lipoproteins such as very low density lipoprotein (VLDL) across plasma membranes, and into cells (*e.g.*, monocytes, cardiomyocytes and adipocytes) for fatty acid oxidation and lipid deposition[17]. Cardiomyocytes use glucose in addition to fatty acids for cellular respiration, and the expression of glucose transporter 4 (GLUT4) and the fatty acid transporter CD36 is stimulated by raised insulin concentrations and an increase in cardiac work[18].

Given that abnormalities of increased CD36 expression and NEFA uptake may represent a common cause for diabetes and the progression of its complications[19-21], we measured changes in the expression of CD36 on circulating monocytes and it association with direct indices of NEFA metabolism amongst South Asians during an oral glucose challenge.

**MATERIALS AND METHODS**

***Participants***

Healthy South Asian volunteers were recruited between Jan and July 2011 for diabetes research (Diabetes Health, Residence and Metabolism in Asians: the DHRMA study - a blinded, randomised, placebo controlled trial) at Sandwell and West Birmingham Hospitals NHS Trust (Birmingham United Kingdom) (described in detail elsewhere[22]). All the participants were aged 18 years and over and were of self reported South Asian ethnicity with no known cardiovascular disease or associated medications, body-mass index (BMI) less than 30 kg/m2, and normal glucose tolerance. The study design was approved by the Local Research Ethics Committee. Written informed consent was collected from all participants.

***Variables***

All patients and controls attended a baseline assessment for the DHRMA study at Sandwell and West Birmingham Hospitals. The assessment incorporated an interview-administered medical history questionnaire, and a dietary assessment (24-h food recall) scrutinised using the WISP (Weighed Intake Software Program) nutritional package (version 3, Tinuviel Software, Llanfechell, United Kingdom[6], and anthropometric measurements, which were measured using Seca scales and stadiometer (Seca Ltd, Birmingham). Waist measurements were recorded from the narrowest circumference above the umbilicus and below the rib, and the hip was recorded as the widest circumference at the buttocks. Both girths were measured in duplicate, and repeated where there were differences more than 2%. Blood pressure (BP) measurement was repeated three times, 1 minute apart (analysing the mean) using a semi automated blood pressure monitor, the OMRON 705CP (Omron Healthcare Europe, Mannheim, Germany) in combination with suitable cuff sizes for each participant, after at least five minutes in the sitting position. During this assessment venepucture was performed to collect blood (as serum, fluoride oxalate plasma and EDTA plasma) at fasting, 30 min post administration of a 75 g oral glucose load (Maxijul; SHS Supplies, Liverpool, United Kingdom). Blood collected with fluoride oxalate was analysed for glucose (glucose oxidase method) within 2 h of venepuncture using the Cobas Integra 400 auto analyser (Roche Diagnostics, United Kingdom). EDTA plasma was stored at 4 oC was analysed by: (1) Flow cytometry was recorded using the BD FACSCalibur flow cytometer [Becton Dickinson (BD), Oxford, United Kingdom] (described elsewhere[23]). Absolute counts of monocytes analysed for human CD36 antibodies (Miltenyi Biotec GmbH, Germany). Monocyte CD36 was also assessed in subsets of monocytes by order of their co-expression of CD14, CD16 and CCR2, defined as Mon1:CD14(+)CD16(-)CCR2(+), Mon2: CD14(+)CD16(+)CCR2(+), and Mon3: CD14(low)CD16(+)CCR2(-) (as described previously[23]); and (2) Density gradient ultracentrifugation (described elsewhere[24]) was used to separate VLDL, low density lipoprotein (LDL), high density lipoprotein (HDL)2 and HDL3 subfractions using the Optima TLX Ultracentrifuge (Beckman Coulter, High Wycombe UK). Briefly, VLDL subfractions were extracted at a density 1.006 kg/L, LDL at 1.063 kg/L, HDL2 at 1.123kg/L and HDL3 at 1.21 kg/L. Concentrations of cholesterol and triglyceride were measured on these separated lipoprotein subfractions on the Cobas Integra 400.

Blood collected as serum was separated and stored at -70 oC for batch analysis using commercially availible (1) colourimetric assays for NEFA (Acyl CoA synthase/oxidase method, Randox Laboratories, Co Antrim, United Kingdom); (2) ELISA for Insulin (Abcam, Cambridge, United Kingdom), adiponectin (R and D systems, Abington, United Kingdom), soluble CD36 (Adipo Bioscience Inc., Santa Clara, United States); and (3) automated biochemistry assays for total cholesterol, HDL cholesterol, LDL cholesterol, apolipoprotein AI, apolipoprotein B on the Cobas Integra 400.

***Statistical analysis***

Statistical review of the study was performed by a biomedical statistician. Data were analysed and validated using SPSS version 16.0 (SPSS Inc., Chicago, IL, United States). The parametric distribution of variables was scrutinised against Kolmogorov–Smirnov plots. Normally distributed data was analysed using ANOVA. The central tendencies of the data were presented as mean and variation by standard deviation (SD). Non-parametrically distributed data were analysed using the Friedman test for related measures, and data were presented as both median and interquartile ranges. A two-tailed bivariate correlation analysis was performed using Spearman’s correlation coefficient. Categorical data were analysed using χ2 tests. A *P* value < 0.05 was accepted as statistically significant.

Statistical review was performed by Dr. Andrew Blann, a biomedical statistician.

**RESULTS**

A total of 29 volunteers were consecutively recruited for this study. The characteristics of the cohort are shown in Table 1. South Asians were typically of Indian origin (72.4%) and subscribed to diets where the fat intake was 40% of the total energy intake. Their mean age was 34.6 (8.9) years, 16 were male (76.2%) and mean levels of BMI, fasting blood glucose, fasting serum lipids, and blood pressure were reflective of a healthy cohort (Table 2). Soluble levels of CD36 were unrelated to monocyte expression of CD36. Analysing variables reported in Tables 1 and 2, variations in the percentage expression of monocyte CD36 was associated with anthropometry and dietary intake (Table 3). These correlations were specific to monocyte subsets, where Mon1 was positively associated with carbohydrate intake, and negatively with fat intake and BMI, while such trends appeared reversed in Mon2 and Mon3 subsets, which were additionally associated with apolipoprotein AI and NEFA (Table 3).

On analysis of serial measures of monocyte CD36 expression, there were evident changes in receptor concentrations for all monocytes (*P* < 0.001), as well as across subsets Mon1 (*P* < 0.001), Mon2 (*P* = 0.011) and Mon3 (*P* = 0.03) (Table 4). The profile of monocyte subset changes reflected a decrease post-prandially (30 min after the glucose challenge) and higher levels post-absorbatively (after 120 min) in Mon1 and Mon2 (Figure 1). NEFA levels progressively decreased during the glucose challenge (*P* < 0.001), whereas cholesterol concentrations within VLDL and LDL subfractions increased (*P* < 0.001). The levels of triglyceride within VLDL particles and HDL particles appeared to decrease during the glucose challenge, but these changes were not significant. Levels of serum triglycerides, glucose, HDL subfraction lipids were largely unchanged following the glucose load. Similarly, there were no significant changes in percentage expression of monocyte CD36 on Mon1, Mon2 or Mon3.

**DISCUSSION**

Greater CD36 expression following a glucose challenge in healthy South Asians, could reflect a physiological response to counteract the toxicity of excessive plasma glucose. Monocytes have been shown to present an intracellular CD36 pool[25], and the transient expression of CD36 in monocytes during glucose challenge, may serve as a critical process in dictating the functional activity of CD36 during diabetic conditions and perhaps, atherogenesis. These increases in absolute monocyte CD36 concentrations occurred in parallel to an exponential decrease in NEFA, a rise in levels of VLDL and LDL cholesterol, and no changes in plasma glucose, serum triglycerides or HDL cholesterol. It is possible that the oral glucose challenge in South Asians results in the generation of VLDL particles, and the increased availability and action of CD36 results in the liberation of triglyceride from these particles. The expression of CD36 on monocytes is associated with factors such as dietary fat and carbohydrate, and we are tempted to speculate that lifestyle exposures have a role in moderating the expression of CD36.

An increase in the expression of CD36 is seen as a dysfunctional event, and in diabetics it is associated with a down regulation of the GLUT 4 receptor and a reduction in glycogen synthesis[18]. In South Asians, this response to increase CD36 expression following a glucose load may reflect a homeostatic process to up-regulate those mechanisms that preferentially store energy as fat. In animal models and cellular models, CD36 is shown to lower circulating NEFA concentrations and to promote the efflux of triglyceride from VLDL[16].

The reasons this “ethnic” preference to process energy in this way are complex. One interesting aspect of CD36 measurement in South Asians is in its dual role in the clearance of red blood cells infected with plasmodium falciparum. The interaction between malaria and CD36 receptors is complex and the subject of debate[26]. South Asians have evolved from a part of the world where malaria was endemic[27], and this may have conferred a survival benefit[28,29].

Hepcidin is upregulated in malarial infected individuals by IL-6[29], a protein that supports hepatic gluconeogenesis, by a process that is dysregulated in diabetics. Further research is required to fully understand the link between exposure to malaria and a subsequent susceptibility to diabetes.

Monocytes and macrophages play a fundamental role in the pathogenesis of atherosclerosis, and various subtypes of monocytes are associated with cardiovascular diseases[30]. Functional differences of three Mon1 Mon2 and Mon3 monocyte subsets have been described[23]. Each monocyte subset responds differently to distinctive immunological stimuli. For example, Mon 2 and 3 monocytes predominate during inflammatory states and Mon 1 in response to Candida albicans[29]. The production of cytokines such as TNF-alpha, associated with diabetes, also varies with stimulus[31]. However, responses in CD36 expression following the glucose challenge were largely similar across these subsets, and as such the metabolic significance of these subclassifications are unclear. The increased surface expression of CD36 on Mon1 was associated with lower BMI and a higher intake of carbohydrate, where as the converse appeared true for Mon1 and Mon2, which were positively associated with dietary fat. Such findings suggest that the expression of CD36 on monocytes can be moderated by diet.

Due to the low numbers of subjects in this study, we only had sufficient statistical power to detect significant associations (alpha at 0.02) where the correlation coefficient was ≥ 0.55. We found levels of soluble CD36 to be unrelated to monocytes and metabolic measures. However, elsewhere, soluble CD36 has been shown to reflect several aspects of insulin resistance in humans[32]. The isolation of lipoprotein subfractions by ultracentrifugation is prone to losses during the extraction process. Further work would need to be undertaken to analyse the generalisability of the data generated here. There is considerable diversity within the South Asian ethnic category, it encompases over 22 different languages and more than 6 different religions. The dietary intake of fat in this cohort was similar to that we have measured in a larger cohort of South Asians Living in the United Kingdom[33]. Nonetheless, the dietary intake of fat in these groups is much greater than that seen amongst South Asians living in rural India[28].

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. We presume that triglycerides from these particles are cleared by an increase in the availability of CD36 receptors, and it would appear that lifestyle exposures may influence this process.

**COMMENTS**

***Background***

The incidence of diabetes in South Asian populations, including those living in the Indian Subcontinent, and those who have migrated away, is significant. Indeed it is higher than in comparable Caucasian populations and the resulting complications of diabetes such as nephropathy and retinopathy occur both earlier and more severely. Despite extensive investigation, the underlying pathophysiological processes explaining this phenomenon remain elusive. For its part, this study investigated lipid and lipoprotein metabolism and its relationship with the expression of the fatty acid translocase CD36 on monocytes in South Asians.

***Research frontiers***

Gene variants in CD36, a macrophage scavenger receptor, have been implicated in the pathogenesis of type 2 diabetes and its complications.

***Innovations and breakthroughs***

This study found that following the administration of a glucose load to South Asian individuals, an upregulation of CD36 positive monocytes was demonstrated which has not been previously seen. In keeping with animal models, it is presumed this increased CD36 expression facilitates the sequestration of triglycerides from VLDL particles.

***Applications***

This finding suggests that CD36 may represent an as yet unexploited target for therapeutic interventions addressing both diabetes and dyslipidaemia.

***Peer-review***

This is a very interesting study and all the sections of the manuscript are complete; results have been well described in the text and in the Tables and Figure.

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**Table 1 Characteristics of South Asian volunteers**

|  |  |
| --- | --- |
| Characteristics of 29 South Asian volunteers | Mean (SD)  |
| Age (yr) | 34.6 | (8.9) |
| Weight (kg) | 69.6 | (18.4) |
| Body-mass index (kg/m2) | 25.0 | (5.2) |
| Waist circumference (cm) | 86.8 | (16.3) |
| Hip circumference (cm) | 101 | (10) |
| Hip to waist ratio | 0.892 | (0.144) |
| Systolic BP (mmHg) | 119 | (13) |
| Diastolic BP (mmHg) | 75.8 | (8.8) |
| Energy from carbohydrates (%) | 45.0 | (6.3) |
| Energy from fats (%) | 40.8 | (5.8) |
| Energy from protein (%) | 9.4 | (6.8) |
| Energy from sugars (%) | 12.6 | (7.5) |
| Energy from starch (%) | 32.0 | (8.5) |
| Energy from saturated fat (%) | 9.7 | (5.8) |
| Energy from monounsaturated fat (%) | 10.7 | (5.2) |
| Energy from polyunsaturated fat (%) | 6.8 | (2.9) |
| Energy from alcohol (%) | 0.5 | (2.2) |

BP: Blood pressure.

**Table 2 Fasting metabolic indices and monocyte CD36 expression amongst South Asian volunteers**

|  |  |
| --- | --- |
| Characteristics of 29 South Asian volunteers | Median (interquartile range) |
| Plasma glucose (mmol/L) | 5.09 | (4.50, 5.47) |
| Serum NEFA (mmol/L) | 0.416 | (0.264, 0.514) |
| Serum cholesterol (mmol/L) | 3.41 | (2.95, 3.96) |
| Serum triglycerides (mmol/L) | 0.90 | (0.67, 1.44) |
| HDL cholesterol (mmol/L) | 1.27 | (0.98, 1.48) |
| LDL cholesterol (mmol/L) | 2.03 | (1.56, 2.44) |
| Apolipoprotein AI (g/L) | 1.21 | (1.10, 1.43) |
| Apolipoprotein B (g/L) | 0.58 | (0.46, 0.77) |
| VLDL subfraction cholesterol (mmol/L) | 1.26 | (0.97, 1.44) |
| LDL subfraction cholesterol (mmol/L) | 1.30 | (0.80, 1.65) |
| HDL2 subfraction cholesterol (mmol/L) | 0.58 | (0.41, 0.76) |
| HDL3 subfraction cholesterol (mmol/L) | 0.37 | (0.25, 0.51) |
| VLDL subfraction triglyceride (mmol/L) | 0.34 | (0.24, 0.43) |
| LDL subfraction triglyceride (mmol/L) | 0.27 | (0.17, 0.55) |
| HDL2 subfraction triglyceride (mmol/L) | 0.08 | (0.06,0.15) |
| HDL3 subfraction triglyceride (mmol/L) | 0.06 | (0.04, 0.08) |
| Insulin (mU/L) | 4.64 | (2.60, 8.23) |
| Adiponectin (ng/mL) | 1.79 | (0.65, 2.65) |
| Plasma soluble CD36 (mmol/L) | 108 | (0, 249) |
| Total Monocyte CD36 (Mon CD36+ per uL)  | 361 | (301, 432) |
| Monocyte CD36 on Mon1 (%) | 81.8 | (75.1, 85.8) |
| Monocyte CD36 on Mon2 (%) | 7.2 | (5.0, 10.3) |
| Monocyte CD36 on Mon3 (%) | 10.5 | (8.7, 16.6) |

Monocyte subset. Mon1:CD14(+)CD16(-)CCR2(+), Mon2: CD14(+)CD16(+)CCR2(+), and Mon3: CD14(low)CD16(+)CCR2(-).NEFA: Non-esterified fatty acids; VLDL: Very low density lipoprotein; LDL: Low density lipoprotein; HDL: High density liporprotein.

**Table 3 Correlation between characteristics and fasting metabolic indices against percentage monocyte CD36 expression**

|  |  |
| --- | --- |
| Characteristics and fasting metabolic indices | Spearman correlation coefficient (*P*) |
| Mon1 | Mon2  | Mon3 |
| Body-mass index | -0.438 (0.02) | 0.156 (0.43) | 0.418 (0.027) |
| Hip circumference | -0.633 (0.002) | 0.419 (0.06) | 0.483 (0.027) |
| Serum NEFA | -0.349 (0.06) | 0.151 (0.43) | 0.478 (0.009) |
| Apolipoprotein AI | -0.282 (0.14) | 0.475 (0.009) | 0.149 (0.44) |
| Energy from total fat (%) | -0.472 (0.036) | 0.399 (0.08) | 0.458 (0.04) |
| Energy from monounsaturated fat (%) | -0.691 (< 0.001) | 0.724 (< 0.001) | 0.230 (0.33) |
| Energy from carbohydrates (%) | 0.645 (0.002) | -0.496 (0.026) | -0.533 (0.016) |

Monocyte subset. Mon1:CD14(+)CD16(-)CCR2(+), Mon2: CD14(+)CD16(+)CCR2(+), and Mon3: CD14(low)CD16(+)CCR2(-).NEFA: Non-esterified fatty acids.

**Table 4 Changes in metabolic factors absolute monocyte CD36 expression pre and post administration of oral glucose**

|  |  |  |
| --- | --- | --- |
|  | Circulating concentrations following the administration of glucose | *P* |
|  | 0 min | 30 min | 120 min |
| Monocyte CD36 on Mon1 (Mon CD36+ per uL) | 300 | (240, 324) | 223 | (187, 254) | 353 | (323, 382) | < 0.001 |
| Monocyte CD36 on Mon2 (Mon CD36+ per uL) | 23.9 | (17.9, 32.3) | 21.3 | (17.2, 30.8) | 29.9 | (18.1, 39.6) | 0.011 |
| Monocyte CD36 on Mon3 (Mon CD36+ per uL)  | 36.7 | (24.3, 53.2) | 26.1 | (18.4, 45.8) | 34.4 | (31.3, 48.7) | 0.03 |
| Total Monocyte CD36 (Mon CD36+ per uL)  | 361 | (308, 432) | 278 | (240, 324) | 422 | (394, 458) | < 0.001 |
| Plasma glucose (mmol/L) | 5.09 | (4.5, 5.47) | 6.68 | (4.93, 8.12) | 5.2 | (4.64, 6.07) | 0.08 |
| Serum NEFA (mmol/L) | 0.416 | (0.264, 0.514) | 0.215 | (0.098, 0.326) | 0.094 | (0.071, 0.207) | < 0.001 |
| Serum cholesterol (mmol/L) | 3.41 | (2.95, 3.96) | 3.29 | (2.95, 3.95) | 3.44 | (3.06, 3.80) | 0.52 |
| Serum triglycerides (mmol/L) | 0.9 | (0.67, 1.44) | 0.83 | (0.55, 1.19) | 1.01 | (0.54, 1.35) | 0.26 |
| VLDL cholesterol (mmol/L)1 | 1.26 | (0.97, 1.44) | 1.31 | (0.94, 1.50) | 1.42 | (1.27, 1.62) | 0.001 |
| LDL cholesterol (mmol/L)1 | 1.39 | (0.89, 1.71) | 1.61 | (0.8, 1.82) | 1.92 | (1.58, 2.24) | 0.003 |
| HDL2 cholesterol (mmol/L)1 | 0.600 | (0.425, 0.735) | 0.470 | (0.360, 0.725) | 0.540 | (0.415, 0.820) | 0.19 |
| HDL3 cholesterol (mmol/L)1 | 0.395 | (0.285, 0.505) | 0.330 | (0.205, 0.450) | 0.325 | (0.250, 0.570) | 0.43 |
| VLDL triglyceride (mmol/L)1 | 0.338 | (0.223, 0.440) | 0.263 | (0.213, 0.365) | 0.265 | (0.105, 0.353) | 0.37 |
| LDL triglyceride (mmol/L)1 | 0.270 | (0.170, 0.505) | 0.250 | (0.170, 0.360) | 0.275 | (0.125, 0.385) | 0.16 |
| HDL2 triglyceride (mmol/L)1 | 0.080 | (0.055, 0.138) | 0.070 | (0.043, 0.093) | 0.060 | (0.028, 0.100) | 0.09 |
| HDL3 triglyceride (mmol/L)1 | 0.058 | (0.035, 0.083) | 0.035 | (0.013, 0.045) | 0.038 | (0.025, 0.065) | 0.12 |

1Subfraction lipids. Data are median (interquartile range). NEFA: Non-esterified fatty acids.



**Figure 1 Changes in monocyte subset CD36 expression** (Mon CD36+ per uL) **pre and post administration of oral glucose.** Monocyte subsets. Mon1:CD14(+]CD16(-]CCR2(+]; Mon2: CD14(+]CD16(+]CCR2(+]; and Mon3: CD14(low]CD16(+]CCR2(-].