Title Page: Physical activity and lipidomics in a population at high risk of type 2 diabetes mellitus

Running title – Physical activity intensity and lipidomics

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Abstract

The aim was to investigate how measurements of the lipidome differ according to the level and intensity of physical activity in a population at high risk of type 2 diabetes (T2DM). A targeted metabolomics platform provided quantitative molecular data on lipid species. Linear regression examined the associations between plasma lipid concentrations, particle size and time spent in objectively measured physical activity intensity domains, in increments of 500 counts per minute (cpm) (up to >4500cpm (~>5.6METs)). Results are presented as % difference in the concentration (lower/higher) or particle size (smaller/larger) per 10 minutes of activity within each intensity. 509 participants were included. Time spent in the lowest physical activity intensity domain (<500cpm) was unfavourably associated with VLDL (2%), HDL (-2%) and Apolipoprotein A-1 particle concentrations (-2%) and HDL diameter (-2%). Conversely, time spent in intensities ≥1000cpm were favourably associated with HDL subclass concentrations; with stronger associations seen at moderate intensities (2000-3999cpm (~4.5METs)). For Apolipoprotein-B concentration and VLDL particle concentration and size, a negative association was consistently observed at the highest physical activity intensity only. If these associations are causal, HDL subclasses appear sensitive to light-intensities whereas only the high category of physical activity intensity was consistently associated with VLDL subclasses.

Keywords: Metabolomics, lipidomics, physical activity, high risk, accelerometer

Introduction

Previous research has consistently demonstrated that individuals who engage in physical activity, particularly moderate-to-vigorous intensity (MVPA), on a regular basis manifest a myriad of physiological benefits related to lipid metabolism (1). For example, HDL-C (including very large HDL particle concentrations) is generally responsive to physical activity and increases in a dose-dependent manner with increased energy expenditure (2,3). Conversely, physical inactivity (the failure to achieve the minimum activity recommendations for health (4)), and sedentary behaviour (any sitting or reclining activity with low energy expenditure (5)) are each independently associated with an increased risk of cardiovascular and all-cause mortality (6,7), primarily driven by a worsening of atherogenic dyslipidemia, which includes reduced HDL-C and so potentially greater non-HDL-C levels (7). In contrast, the impact of exercise and inactivity on LDL-C, triglycerides and triglyceride rich lipoproteins are less consistent (8).

Lipidomics is a sub-class of metabolomics focussing on the structure and function of lipids and lipid derivatives (e.g. phospholipids). These molecules may aid in pinpointing the molecular pathways linking health and disease and how they are influenced by lifestyle behaviours, such as physical activity (9). Historically, many studies have focused exclusively upon the metabolite response to exercise training (10,11). More recently, studies have also reported associations in relation to habitual physical activity and sedentary behaviour across multiple metabolite networks (3,12,13).

However, there has been limited research on lipidomics and physical activity in populations at high risk of chronic disease. This is an important limitation as international recommendations and policies specify that chronic disease prevention strategies should include targeted interventions aimed at the identification and management of high-risk individuals (14,15). Therefore, the importance of sedentary behaviour and physical activity in this group needs to be better understood in order to inform the content and structure of prevention programmes. Moreover, previous investigations have typically categorised sedentary behaviour and physical activity (light, moderate, vigorous) using population-dependent thresholds. Using a broader continuum of intensity categories allows for greater insight into the dose–response relationship between physical activity intensity and health outcomes (16). This is important as previous research has typically focused on MVPA, which occupies a very

small fraction of the day, if at all. Conversely, substantial cardiometabolic benefits may be gained from light-intensity activity, particularly in those at high risk of chronic disease (17), which may also represent a more feasible means to increasing overall activity volume. Applying this approach to measurements of the lipidome may allow for greater understanding of how lipid metabolism differs across the precise physical activity intensity spectrum.

Therefore, the aim of this study was to explore the associations between circulating lipid species and various physical activity intensities in a population at high risk of type 2 diabetes mellitus (T2DM).

Materials and Methods

Study population

This study reports cross-sectional baseline data from the Walking Away from Diabetes study. Participants were recruited through 10 primary care practices in Leicestershire, UK (18). Individuals at increased risk of impaired glucose regulation (IGR; any combination of impaired glucose tolerance (IGT) and/or impaired fasting glycaemia (IFG) or undiagnosed T2DM) were identified for recruitment using a modified version of the Leicester Risk Score (19). This risk scoreapplies a validated algorithm to routinely collected data within primary care; based on age, sex, BMI, ethnicity, prescribed antihypertensives, and family history of diabetes. Those individuals scoring within the 90th percentile in each practice were invited to take part in the study. This approach has reasonable sensitivity and specificity for identifying participants with IGR (19). Individuals were unaware of their diabetes risk status before entering the study. Those who had previously been diagnosed with T2DM, were currently taking steroids or were unable to take part in any walking were excluded. Written informed consent was obtained from all eligible participants and the study had full ethical and governance approval.

Accelerometer derived measures of physical activity

All eligible participants were asked to wear an accelerometer, (Actigraph GT3X, Florida, USA), around their waist, for seven consecutive days during waking hours. These accelerometers translate raw accelerations into activity counts. Data were recorded in 15-s epochs and reintegrated into 60-s epoch files for this analysis. Non-wear time was defined as a

minimum of 60 minutes of continuous zero counts; days with at least 600 minutes of wear time were considered valid. In order to be included in the analysis, participants required a minimum of any four valid days (20).

A commercially available data analysis tool (KineSoft version 3.3.76, Kinesoft, Loughborough, UK; www.kinesoft.org) was used to process the accelerometer data. Activity intensity was generated in increments of 500 counts per minute (cpm) from 0 to 4499cpm for each participant who met the inclusion criteria for accelerometer wear time; with the corresponding categories (0-499, 500-999, 1000-1499, 1500-1999, 2000-2499, 2500-2999, 3000-3499, 3500-3999, 4000-4499) representing a summation of all included individuals. Any counts above 4500 were amalgamated, due to a lack of power at higher intensities. For descriptive purposes and to aid interpretation, we used the following thresholds to group 500 cpm increments into: very low intensities of physical activity or sedentary behaviour (<500cpm); light-intensity physical activity (≥500-<2000cpm) and MVPA (≥2000 counts per minute); these thresholds were are broadly comparable to those that have been commonly used in the literature (21-23).

Blood sample collection and lipidomics analysis

Lipids were measured from Ethylenediaminetetraacetic acid (EDTA) plasma samples, obtained following an overnight fast and avoidance of alcohol and MVPA for 48 hours previously. The level of systemic lipids in the fasting state arise from a broad combination of genetic and lifestyle related factors. As such, the nuclear magnetic resonance (NMR) spectroscopy metabolomics platform provides a comprehensive snapshot of the individual's physiological status as reflected in their systemic metabolism (9).

Analysis was performed by Nightingale Health (Helsinki, Finland), whose platform and procedures have been described elsewhere (9). Given the fact that the chosen NMR spectra allows significant modelling of lipoprotein subclasses (24), coupled with the previous epidemiological work showing associations between sedentary time, physical activity and cardiovascular outcomes (6,20,25), the targeted focus of our analysis was on lipid species.

Briefly, plasma samples were analysed using an automated high-throughput NMR workflow, acquiring NMR spectra on either a Bruker AVANCE III 500 MHz or Bruker AVANCE III HD

600 MHz spectrometer. Following organic solvent lipid extraction, further NMR spectra were acquired from the lipid extracts on the 600 MHz spectrometer. The initial data processing, including the Fourier transformations to NMR spectra and automated phasing were performed using computers that control the spectrometers. The spectra were then automatically transferred to a centralized server, which performs various automated spectral processing steps, including overall signal check for missing/extra peaks, background control, baseline removal and spectral area-specific signal alignments (9). The spectral information also underwent various comparisons with the spectra of 2 quality control samples; the data for which is also followed and compared in a consecutive manner. For those spectral areas that passed all the quality control steps, regression modelling was performed to produce the quantified molecular data. Individual metabolic measures also underwent various statistical quality control steps and were checked against an extensive database of quantitative molecular data (9). All analyses were conducted by individuals blinded to the participants' identity and physical activity levels. As traditional clinical lipid profile may not fully capture meaningful information with regards to cardiometabolic risk (26), we report the concentration of particles ("number") within subclasses of VLDL, HDL, IDL and LDL, apolipoprotein concentration (Apolipoprotein-A1 (Apo-A1) and Apo B) and the ratio of Apo B to Apo-A1. We also report the mean diameter particle size of VLDL, HDL and LDL.

Covariates

Information on smoking status and ethnicity was obtained following an interview administered protocol conducted by a healthcare professional. We were also able to adjust for available dietary biomarkers (omega-3 and omega 6 fatty acids) which are reflective of the composition of ingested fatty acids (27) and act as lipid mediators in the inflammatory response (28). In addition, an increasing dietary ratio of omega-3/omega 6 fatty acids has been associated with a higher incidence of obesity, cardiovascular disease (CVD), metabolic syndrome and insulin resistance (28-30). Conversely, diets including high amounts of seafood and fish increase the dietary amount of omega-3 and have been linked to a reduced risk of CVD, T2DM and metabolic syndrome (31,32). .

Statistical analysis

Linear regression was used to examine the associations between lipid type concentrations, particle size and physical activity intensities. All lipid outcomes were log-transformed, standardised (Z score) and centered (mean =0, standard deviation =1). Any value that was below detection was set to the minimum observed value of the corresponding lipid. Time spent in each of the physical activity intensity increments was entered into models individually because of the high correlation between intensities (Table S1). Models were adjusted for age (continuous), sex (categorical), smoking status (categorical), ethnicity (categorical),, time accelerometer worn (continuous; average minutes per day) and omega-3 and 6 fatty acids (continuous).

Results are presented as % difference in the lipid variable associated with 10 minutes of each activity within each intensity. Two-tailed *p* values of <0.05 were considered statistically significant. No further adjustment was made for multiple comparisons, therefore data were viewed with caution and in relation to the overall pattern of results. All statistical analyses were conducted using IBM SPSS Statistics v24.0.

Results

A total of 509 participants had complete lipidomic and accelerometer data (63% of total sample). The main reasons for participants not having complete data was insufficient accelerometer wear time over too few days and insufficient volumes of blood for additional analyses. There was no difference in the proportions of males/females, ethnicity, smoking status or age in those included vs. those excluded. Table 1 displays the characteristics of included study participants.

Physical activity

The average time spent in each 500cpm intensity banding is shown in Table 1. 82.4% of total accelerometer wear time was spent in the lowest physical activity category (<500cpm), compared with 0.3% in the highest activity category (>4500cpm). Table S1 also displays the correlations between each intensity band.

Lipoprotein concentrations and particle size

The associations between lipid sub-type particle concentrations and physical activity intensities (in 500cpm increments, per 10 minutes of activity) are displayed in Figure 1 with associations for apolipoprotein concentration displayed in Figure 2 (corresponding values presented in Tables S2 and S3). Figure 3 displays the associations between particle size and physical activity intensities.

Lipoprotein subclass HDL

Concentrations of both large and medium HDL particles showed negative associations with time spent in the lowest intensity of physical activity, which is likely to include a significant amount of sedentary behaviour (<500cpm (both -2%; 95% CI= -3% to -1%, per 10 minutes of activity)) (Figure 1A, Table S3). Time spent in physical activity intensities >1000cpm were favourably associated with small and medium HDL subclasses (range = 3%-24%) with results displaying a dose response relationship for medium subclasses up to moderate intensities. Concentration of very large HDL particles were only associated with time spent in the highest intensity of physical activity (>4500cpm).

Lipoprotein subclass VLDL

Time spent in the lowest physical activity intensity band (<500cpm) was negatively associated with the concentration of very large, large, medium and small VLDL particles (2%; 95% CI= 1% to 3%, per 10 minutes of activity) (Figure 2A, Figure 2C; Table S3). For higher intensities of physical activity, the majority of VLDL subclasses were only found to be favourably associated with time spent in the highest intensity category (>4500cpm).

Concentration of IDL and LDL particles

There was no association between physical activity intensities and IDL particle concentrations, whereas LDL particle concentrations (small, medium and large) were only associated at 3500-3999cpm (range =10%-11%) (Figure 1D; Table S3).

Apolipoproteins

Apo-A1 was negatively associated with physical activity <500cpm (-2%; 95% CI= -3% to -1%, per 10 minutes of activity; Figure 2). Even low levels of activity (≥500cpm) yielded positive associations, with significant results seen up to >4500cpm (range = 3-20%). For Apo B, significant negative associations were seen from moderate (2500-2999cpm) through to the highest physical activity intensity (>4500cpm) (range =-1% to -15%). The ratio of Apo B to Apo-A1 also displayed negative associations as the physical activity intensity increased, with significant results seen at >2500cpm (range = -11% to -23%).

Lipoprotein particle size

Time spent in the lowest intensity of physical activity (<500cpm) was significantly associated with a higher mean diameter of VLDL (2% (95% CI = 1% to 3%, per 10 minutes of activity)) and lower mean diameter of HDL (-2% (95% CI= -3% to -1%, per 10 minutes of activity)) particles (Figure 3; Table S2). As the physical activity intensity increased, there was a dose-response relationship for HDL, with greater intensity associated with a larger particle size, whereas differences in VLDL particle size were observed at the lowest (<500cpm, 500-999cpm, 1000-1499cpm)and highest physical activity intensities (>4500cpm). No associations were seen for the mean diameter of LDL.

Discussion

This study highlights the dose-response associations between physical activity intensity and lipid species involved in the underlying pathophysiology of insulin resistance, CVD and physical activity in a population at high risk of T2DM. The most consistent associations were seen in the HDL and VLDL subclass concentrations. Associations between VLDL subclass concentrations and physical activity were consistently only evident at the lowest (<500cpm, approximately <2.6 METs (33)) and highest intensity of physical activity (>4500cpm, approximately >5.6 METs (33)). Conversely, although results for concentrations of very large HDL particles mirrored those for VLDL, those for smaller HDL particles and Apo-A1 showed significant adverse associations with time spent in the lowest category of physical activity (<500cpm) and positive associations across the spectrum of light- and moderate-intensity physical activity. These results suggest that engaging in different intensities of physical activity

may result in a differential impact on lipid metabolism, with high intensities of physical activity needed to disrupt the hepatic release of VLDL.

To our knowledge, this is the first study to specifically investigate the association of a spectrum of intensities of objectively assessed physical activity on the lipidome, with the findings extending previous research using broad categories of physical activity. For example, a previous study in twins reported that compared to inactive individuals, active individuals had a shift towards lower levels of VLDL and higher levels of large and very large HDL (3). Our findings give insight into how physical activity intensity contributes to these observations, with most HDL subclass concentrations and Apo-A1 being sensitive to time spent in lower intensities of physical activity, whereas lower levels of VLDL subclasses and Apo B were consistently only associated with a moderate to high intensity of physical activity. The results for VLDL and Apo B are consistent with previous research suggesting that the intensity of aerobic exercise must surpass that of moderate intensity in order to have a favourable effect on non HDL-lipids, with adaptations largely modulated through glucagon stimulation (34,35).

For HDL-cholesterol concentrations and Apo A-1 , associations were consistently seen across light and moderate intensities of physical activity up to a threshold of between 2500–3500cpm (approximately 3.7-4.4 METs (33)), after which little additional benefit was observed. This intensity of physical activity is equivalent to walking at ~5km/h and is considered at the lower end of the moderate intensity spectrum (36). Our finding for HDL subclasses is somewhat in agreement with a recently published study of 66 metabolome measures, which found that higher cardiorespiratory fitness, for which moderate intensities of physical activity are an important determinant, was associated with greater concentrations of larger HDL-particles (37). Our findings are also broadly consistent with a meta-analysis of exercise training studies which concluded that duration, and not intensity, is a predictor of the HDL-C response (38). It has also been shown that low-intensity exercise may improve reverse cholesterol transport via the activation of gene transcription variables proliferator-activated receptor gamma (PPARgamma) and liver X receptor alpha (LXRalpha) (39). HDL-cholesterol may also be affected by other physiological processes, such as inflammation (40), which may be influenced by overall volumes of physical activity (41). Therefore, these data suggest that both light-intensity and moderate-intensity physical activity interventions are effective at improving HDL-C concentrations, whereas engaging in higher-intensities of physical activity may not provide additional benefit on HDL-C.

A further novel finding was that the time spent in moderate intensities of physical activity were associated with larger average HDL-C particle size. Larger HDL particles are hypothesised to be more important in promoting health benefits and thus reducing the risk of CVD (42). Therefore, one of the many mechanisms linking moderate physical activity to cardiometabolic health could be through altering HDL particle size. Previous studies have also reported stronger associations between self-reported physical activity status and greater effects of exercise intervention studies on large HDL compared to smaller particles (43). That noted, we accept that recent trials and genetics have placed a question on the causal link between HDL-C and CVD (44).

Our findings also extend previous findings by showing that low levels of physical activity (<500cpm), indicative of sedentary behaviour, are detrimentally associated with HDL concentrations (20,45). Interestingly, the time spent below 500cpm was also detrimentally associated with Apo-A1 and the concentration of very large, large, medium and small VLDL particles. VLDLs are substrates for lipoprotein lipase (LPL)-mediated triglyceride removal, with larger VLDL particles carrying more triglycerides than smaller particles and correlating with insulin resistance (46). Although the precise mechanism of sedentary behaviour and (in)activity-induced lipid changes are unclear, muscle LPL regulation is thought to be one of the most sensitive metabolic responses to sedentary behaviour and low-intensity contractile activity and may explain why even small amounts of physical activity appear to confer cardiovascular benefits (47). The mechanistic relevance of LPL to sedentary behaviour has been demonstrated in animal models (48) whereas in humans moderate intensity activity was shown to increase the affinity of large VLDL particles for LPL clearance (49). However, further insight is needed into the precise impact of increased sedentary time and reduced physical activity on LPL activity.

Strengths and limitations

Strengths of our study include the objective measurement of physical activity and examination of lipids in relation to different characteristics across a range of physical activity intensities. By enabling identification of the minimum intensity at which benefits may occur as well as a quantifiable dose–response relationship, this information may aid in generating hypotheses to be tested in future physical activity interventions. Furthermore, our targeted metabolomic

platform covered a wide variety of lipids with known identity and quantitative measurements. Our results are strengthened by the fact that associations with all lipoprotein subclasses were present after adjusting for dietary biomarkers (omega-3 and omega 6), supporting an independent association of physical (in) activity *per se* on the lipoprotein subclass profile. These results are in agreement with previous studies which have shown that significant changes in HDL and VLDL concentrations and particle size after exercise training are independent of diet (43). Our study is also accompanied by important limitations. For example, despite individuals spending a reasonable amount of time in moderate activity, the time spent in higher, more vigorous intensity activities is limited. However, this is likely reflective of the habitual behaviour of the majority of individuals at high risk of T2DM. This coupled with the fact that our analysis is observational, means that we cannot prove biological mechanisms or demonstrate causality; reverse causality is also a possibility whereby those with a greater burden of risk factors may be less likely to engage in greater volumes or intensities of physical activity The high risk nature of the cohort, where higher relative exercise intensities can be anticipated for a given exercise compared to a healthy population, may also affect the interpretation of the intensity thresholds used for this study. However, this is unlikely to affect the interpretation for HDL-cholesterol, where associations were seen across sedentary time and the lower intensity spectrums. Furthermore, despite adjusting for a range of potential cofounders, residual confounding or confounding from unmeasured factors remains a possibility (e.g. alcohol intake). Finally, although accelerometers allow for more robust assessments of physical activity compared to self-report, they are not without limitations. They rely on categorising movement (acceleration) strength, rather than directly distinguishing between postures or modes of physical activity.

In conclusion, our data suggests potential differences in the associations between different physical activity intensities and the lipidome in subjects with a high risk of T2DM, with most HDL subclass and Apo A-1 concentrations appearing sensitive to light-intensities of physical activity. Although structured physical activity should remain a strong focus and end point of behavioural interventions, lipid related benefits may be gained through light-intensity activity (whilst also reducing sedentary time). Given the limited time spent in higher intensity activities in this population, this may also be the option that is best tolerated in those at high risk of chronic disease. This is particularly pertinent as they are also representative of those likely to be identified as being at high risk of T2DM within routine care and referred onto available prevention programmes. Therefore, future interventions that encourage increases in physical

activity, may need to be tailored to individual characteristics and tolerability. In particular, consideration should be given to the relative intensity of physical activity prescribed, as the absolute values will differ considerably between individuals The results of this analysis also highlight the fact that more work is needed to elucidate the mechanisms by which different physical activity intensities, particularly at the lower end of the spectrum, impact health.

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Conflict of Interest

The authors declare no conflict of interest

Data availability

The datasets generated during and/or analysed during the current study are not publicly available but are available from the corresponding author upon reasonable request.

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Table 1. Participant characteristics

Variable	All
Participants (N)	509
Age (years)	64 ± 8
Female	176 (34.6)
Current smokers	35 (6.9)
Glycosylated haemoglobin (HbA1c) (%)	5.9 ± 0.4
HbA1c (mmol/mol)	41.0 ± 2.0
Total cholesterol (mmol/L)	5.1 ± 1.0
LDL (mmol/L)	3.1 ± 0.9
HDL (mmol/L)	1.4 (0.4)
Triglycerides (mmol/L)	1.3 (0.7)
Ethnicity	
White European	473 (92.9)
South Asian	33 (6.5)
Other	3 (0.6)
Cardiovascular disease*	176 (34.6)
Accelerometer variables (time in minutes per day)	
Wear-time	853.4 ± 84
<500cpm	704.3 (127.4)
500-999cpm	73.3 (40.8)
1000-1499cpm	29.8 (26.5)
1500-1999cpm	13.2 (15)
2000-2499cpm	6.8 (8.66)
2500-2999cpm	4.0 (5.5)
3000-3499cpm	2.5 (4.9)
3500-3999cpm	1.2 (3.8)
4000-4499cpm	0.3 (2.3)
>4500cpm	0 (1.5)
Average steps per day	6581 ± 3143

Data presented as mean ± standard deviation, median (interquartile range) or number (column percent). cpm=counts per minute. *Cardiovascular Disease is defined a medical history of one or more of the following: Myocardial Infarction (MI), Heart Valve Disease, Heart Failure, Atrial Fibrillation, Angina, Stroke, Angioplasty/Coronary Artery Bypass Graft, Leg Angioplasty/bypass, Peripheral Vascular Disease.

Figures

Figure 1a. Forest plot displaying the percentage difference in HDL subclass concentrations with a 10 minute increase in time spent in bands of 500 counts per minute of physical activity intensities.

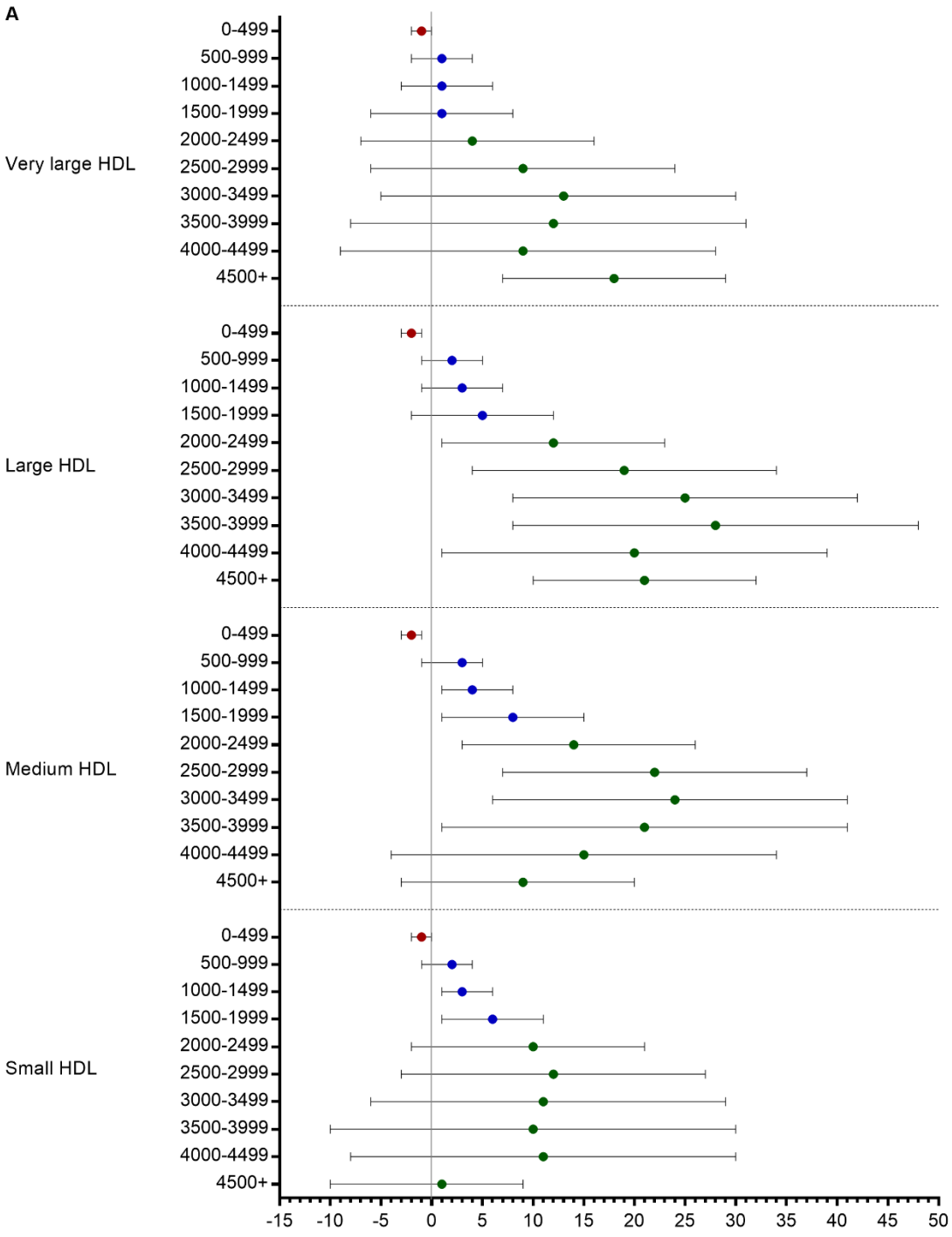


Figure 1b. Forest plot displaying the percentage difference in VLDL subclass concentrations with a 10 minute increase in time spent in bands of 500 counts per minute of physical activity intensities.

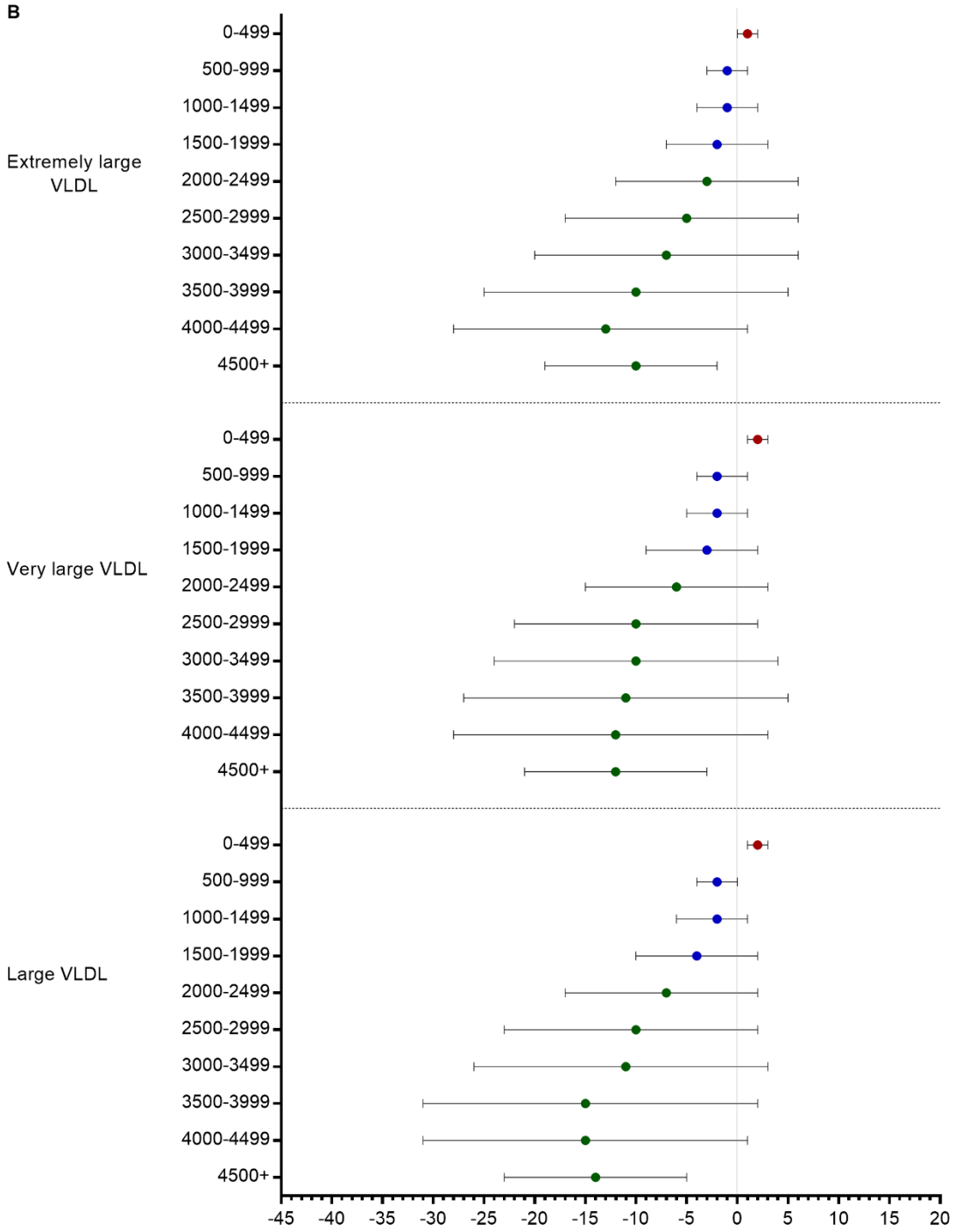


Figure 1c. Forest plot displaying the percentage difference in VLDL subclass concentrations with a 10 minute increase in time spent in bands of 500 counts per minute of physical activity intensities.

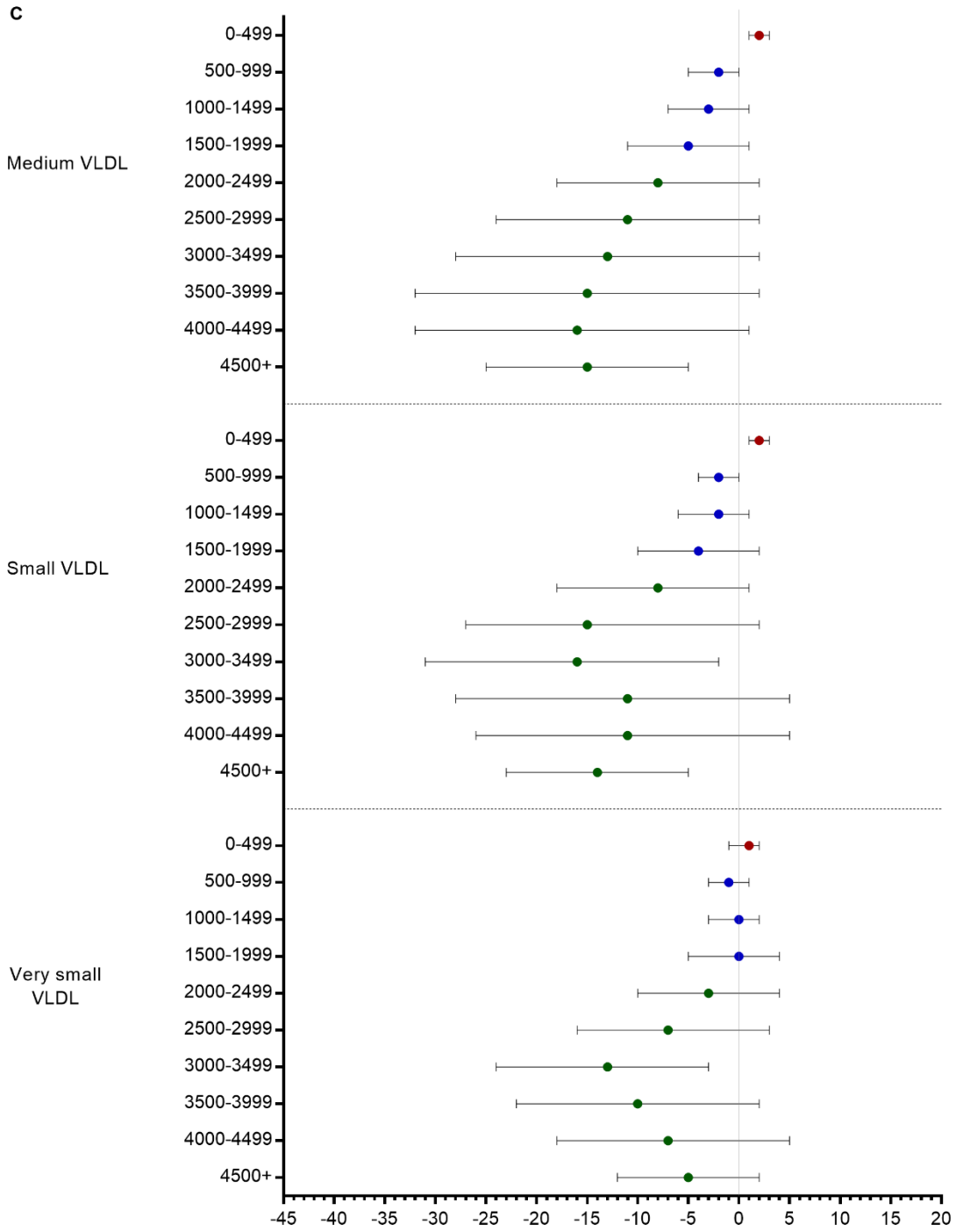


Figure 1d. Forest plot displaying the percentage difference in IDL and LDL subclass concentrations with a 10 minute increase in time spent in bands of 500 counts per minute of physical activity intensities.

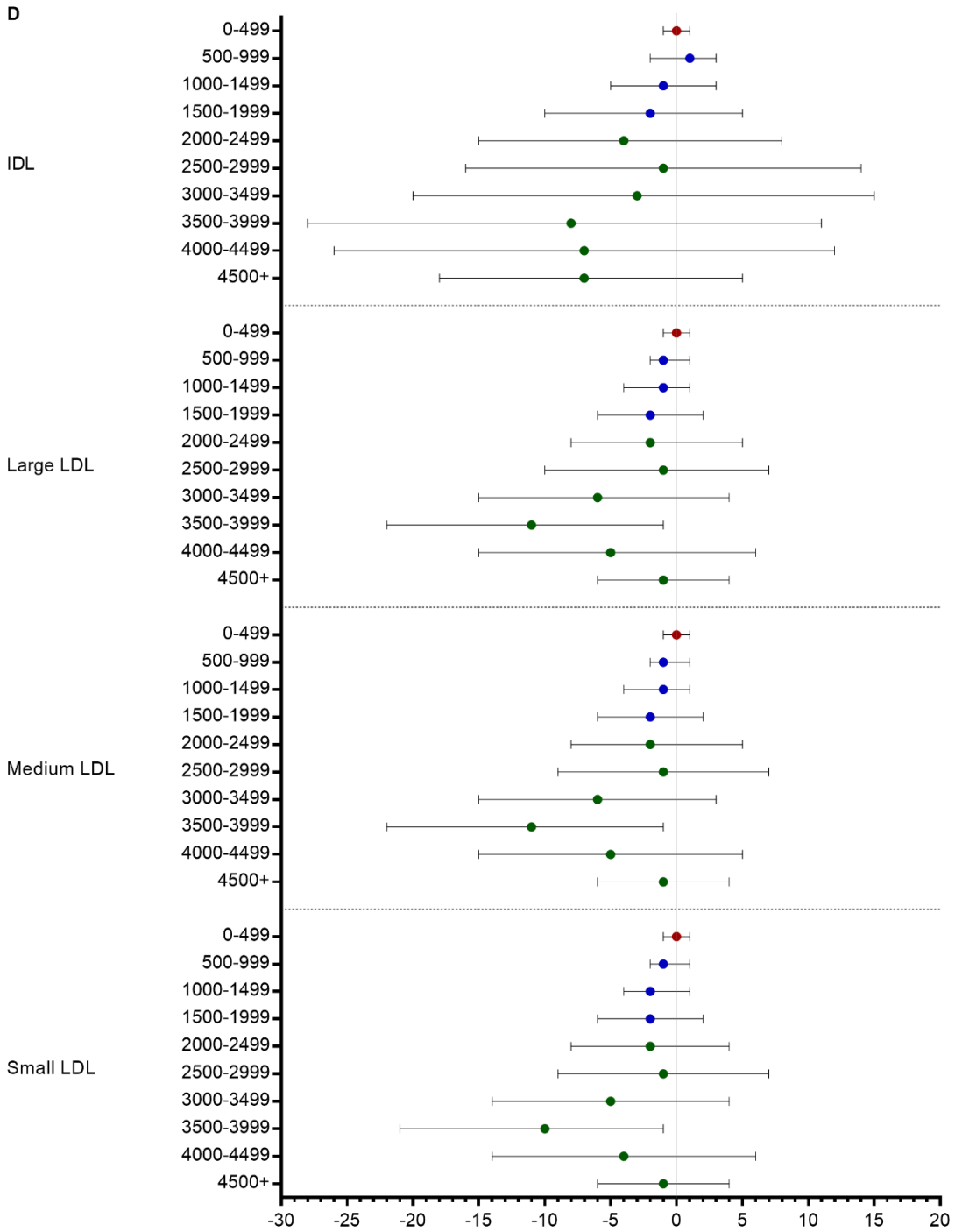


Figure 2. Forest plot displaying the percentage difference in apolipoproteins with a 10 minute increase in time spent in bands of 500 counts per minute of physical activity intensities.

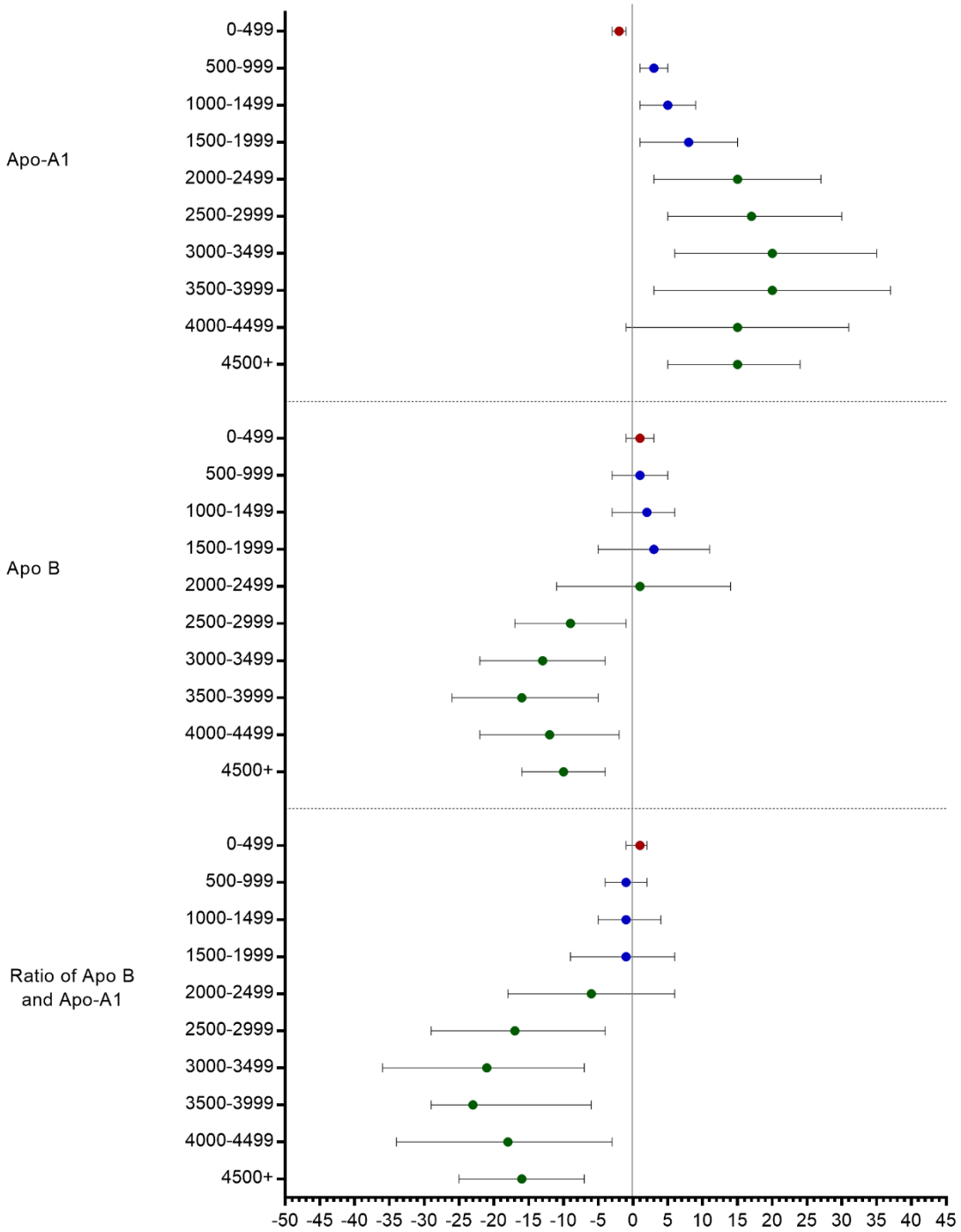
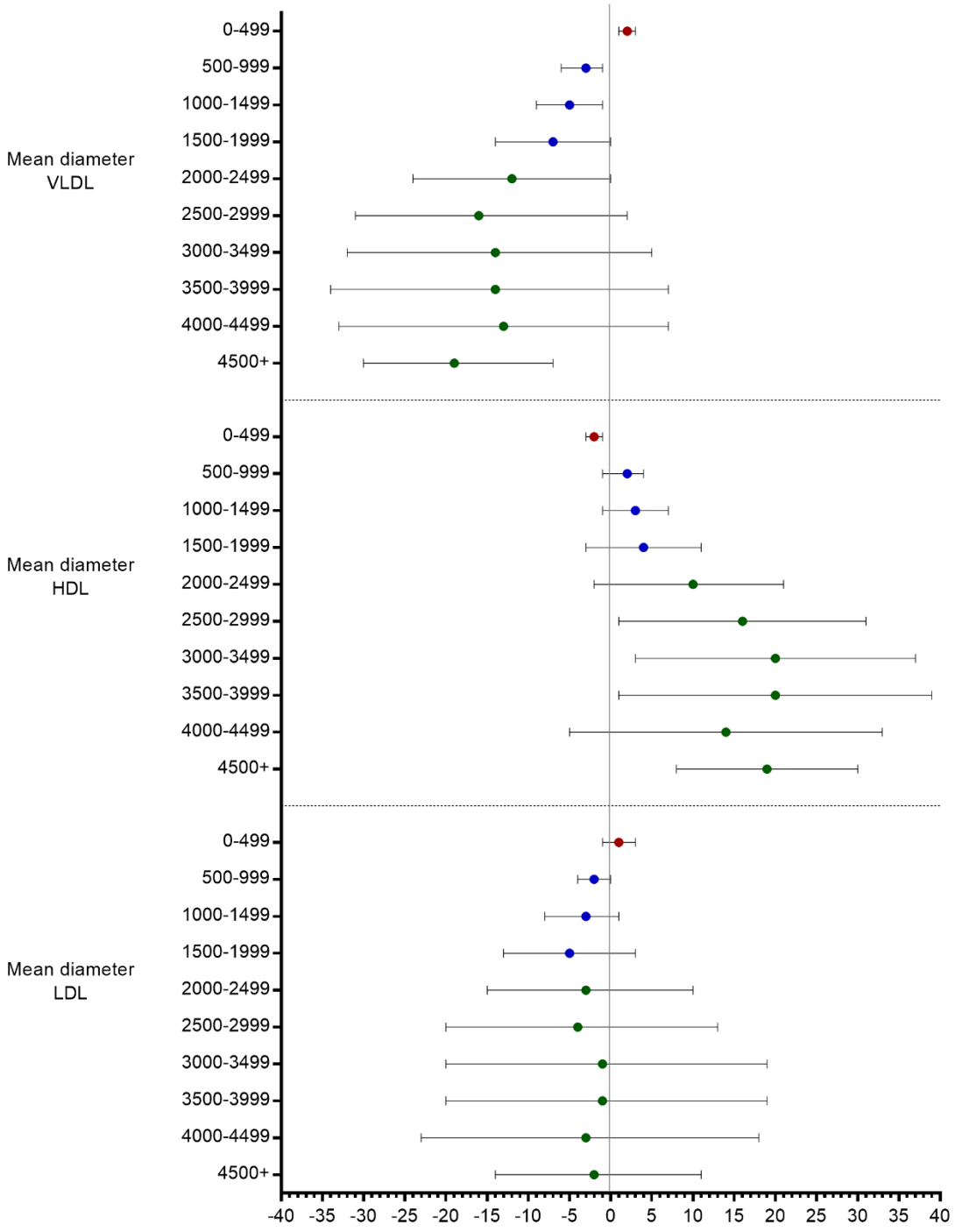


Figure 3. Forest plot displaying the percentage difference in lipoprotein particle size with a 10 minute increase in time spent in bands of 500 counts per minute of physical activity intensities.



Colours broadly represent commonly used accelerometer cut points for low levels of physical activity, which includes sedentary behaviour (red) (<500cpm), light (blue) (≥500-<2000cpm) and MVPA (green) (≥2000 counts per minute).