Associations between weight change and biomarkers of cardiometabolic risk in South Asians: secondary analyses of the PODOSA Trial

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Background/Objectives

The association of weight changes with cardiometabolic biomarkers in South Asians has been sparsely studied.

Subjects/Methods

We measured cardiometabolic biomarkers at baseline and after 3 years in the Prevention of Diabetes and Obesity in South Asians (PODOSA) Trial. We investigated the effect of a lifestyle intervention on biomarkers in the randomised groups. In addition, treating the population as a single cohort, we estimated the association between change in weight and change in biomarkers.

Results

Complete data were available at baseline and 3 years in 151 participants. At 3 years there was an adjusted mean reduction of 1·44kg (95% CI 0.18 to 2.71) in weight and 1.59cm (95% CI 0.08 to 3.09) in waist circumference in the intervention, compared with control, arm. There was no clear evidence of difference between intervention and control arms in change of mean value of any biomarker. As a single cohort, every 1kg weight reduction during follow-up was associated with a reduction in triglycerides (-1.3%, p=0.048), ALT (-2.5%, p=0.032), GGT (-2.2%, p=0.040), leptin (-6.5%, p<0.0001), insulin (-3.7% p<0.001), fasting glucose (-0.8%, p<0.001), 2-hour glucose (-2.3%, p<0.001) and HOMA-IR (-4.5%, p<0.001).There was no evidence of associations with other lipid measures, t-PA, markers of inflammation, or blood pressure.

Conclusions

We demonstrate that modest weight decrease in SAs is associated with improvements in markers of total and ectopic fat as well as insulin resistance and glycaemia in South Asians at risk of diabetes. Future trials with more intensive weight change are needed to extend these findings.

INTRODUCTION

People of South Asian ethnic origin are at increased risk of type 2 diabetes over the full range of body mass indices (BMI), and when living in high-income countries such as the UK, are at higher risk compared to those of White European origin [1]. As such, existing clinical guidelines for the prevention of diabetes have emphasised the importance of targeting lower BMI in this group to mitigate this elevated risk [2,3]. However, clinical trials involving lifestyle interventions have generally only had very modest effects in reducing adiposity among South Asians [4]. For example, although incident diabetes was reduced by lifestyle intervention in the Indian Diabetes Prevention Programme (IDPP-1), absolute BMI and waist circumference increased in all trial arms over 30 months of follow-up [5]. The Prevention of Diabetes and Obesity in South Asians (PODOSA) study of 171 South Asians with impaired glucose tolerance or impaired fasting glucose recently reported that the group who received a culturally adapted lifestyle intervention lost an adjusted mean difference of 1.64kg (95% CI 0.44 to 2.83) in weight and 1.89cm (95% CI 0.52 to 3.27) in waist circumference, compared to the control group [6,7].

It is not known whether a lifestyle intervention resulting in a weight loss of this magnitude will be associated with appreciable changes to metabolic and cardiovascular risk in South Asians. This question is important given the considerable investment of resources required to achieve modest changes in adiposity. The Practice-based Opportunities for Weight Reduction University of Pennsylvania (POWER-UP) trial recently showed that an enhanced intervention that results in a mean 2.9kg weight loss compared to the control arm over 2 years resulted in only small changes in insulin, HOMA and triglycerides [8]. A recent meta-analysis of lifestyle interventions in people with type 2 diabetes found that small reductions in BMI (standardised difference in mean BMI 0.29; 95% CI 0.06 to 0.52) resulted in small reductions in blood

pressure and improved glycaemic control, but no change in lipids [9]. Trials of weight loss interventions have also been performed in people without type 2 diabetes in a range of settings [9]. However, such studies typically have included only small numbers of South Asians. Given the elevated cardiometabolic risks among South Asians, it is important to investigate the effect of lifestyle intervention and associated modest weight loss specifically in this ethnic group.

The aim of the present study was to utilise data from the PODOSA Trial in a planned exploratory analyses to explore the effect of weight loss on the cardiometabolic risk profile over four domains (i.e. lipids, liver function, inflammatory, and metabolic) to address two pre-specified research questions. The first was to determine the effects of the culturally-adapted PODOSA lifestyle intervention [10] on cardiometabolic risk factors using an intention-to-treat analysis. In the second pre-specified analysis, we wished to explore the association of changes in weight and waist circumference with these risk factors using data for all individuals in this trial (i.e. combining intervention and control groups) as a single cohort study.

SUBJECTS AND METHODS

Trial design and participants

PODOSA was a non-blinded, family clustered, randomised controlled trial conducted in Scottish communities, the details of which have been described previously [6,7,11]. In brief, recruitment for screening used a multi-pronged approach (i.e. via the NHS and directly from the community) and took place between 2007 and 2009. Men and women of Pakistani or Indian ethnic origin aged 35 years or older were eligible for screening if their waist size was greater than 90cm and 80cm in men and women, respectively; the screened individual had to

be free of a diagnosis of diabetes; and the family cook was to be willing to cooperate with the trial. Final enrolment was based on confirmed impaired glucose tolerance or impaired fasting glucose based on World Health Organization (WHO) criteria during the screening visit [12]. Families living in the same household, or close to the index participant, aged 18 or over, were randomised to intervention or control arms of the trial as a cluster. Those randomised to intervention were offered information and demonstrations on healthy shopping and cooking practices, and received 15 visits from a dietitian over 3 years of follow-up, who advised on achieving weight loss through calorie deficit and physical activity of at least 30 minutes per day using culturally sensitive techniques. The control group was given standardised written and verbal advice on healthy eating, diabetes prevention, promotion of physical activity, and on accessing other weight control and physical activity services over four visits (baseline, then annually) with a dietitian. In total, 78 families were randomised to each of the intervention and control arms, with 85 and 86 pre-diabetes individuals randomised, respectively.

For all participants, at the baseline and 3-year visits (among others) trained dietitians collected anthropometric data (weight [to the nearest 0.1kg] and height, hip and waist circumferences [to the nearest cm]), blood pressure measurements, and overnight fasted venous blood samples following standard operating procedures for the trial [7]. Weight was measured using SECA 862 digital scales which were calibrated annually. Two unblinded blood pressure measures (Omron M6 BP monitor, calibrated anually) were taken at each visit by study dietitians, and if >10mmHg between either systolic or diastolic then a 3rd measure was performed. The only blinding of group status was at the final measure of weight and waist size, which was performed by independent research nurses. Treatment with medications was based on self-reported (yes/no) responses to questions as to whether participants were

taking statins or anti-hypertensives at baseline and the final visit; data on drug and dose are not available.

Ethical approval

Individual participants gave written informed consent and ethical approval was granted by the Scotland A Multi-Centre Research Ethics Committee.

Biochemical measurements

Biochemical measurements were made by technicians blinded to intervention status at both time points. Measures of high sensitivity C-reactive protein (CRP), total cholesterol, high density lipoprotein (HDL)-cholesterol, triglycerides, gamma-glutamyl transferase (GGT), aspartate aminotransferase (AST), and alanine aminotransferase (ALT) were performed on an automated clinically validated platform (Roche c311, Roche Diagnostics, Burgess Hill, UK). LDL cholesterol was calculated from the Friedwald equation (LDL Cholesterol = Total Cholesterol - HDL Cholesterol - (Triglycerides / 5)). All automated assays were performed using the manufacturer's calibrators and quality control materials. Between run coefficients of variation (CVs) were 6.4% for CRP and <1% for all other assays. Insulin (Ultrasensitive Mercodia ELISA, Diagenics, Milton Keynes, UK), IL-6 and leptin (R&D Systems, Oxon, UK) and tissue plasminogen activator (tPA) antigen (Tcoag TriniLIZE tPA, Stago, Berkshire, UK) were measured by commercially available ELISAs. Intra- and inter-assay CVs for the ELISAs were 4.8% and 6.7% for insulin; 3.9% and 12.6% for IL-6; 7.5% and 6.8% for leptin; and 9.4% and 6.4% for tPA. HOMA-IR was calculated: HOMA-IR = fasting plasma glucose (mmol/l) * fasting serum insulin (μU/l) / 22.5 [13].

Statistical analysis

Analyses were restricted to participants with complete data for all characteristics under study at both baseline and 3 year follow-up. The distributions of each continuous characteristic were examined by randomised group at baseline and 3 years and these were summarised as means (standard deviation [SD]) when normally distributed and median (interquartile range [IQR]) when skewed. Categorical variables were reported as frequencies (percentages). Absolute changes from baseline to 3 year follow up were calculated for weight, waist circumference and all biomarkers. Correlations of weight and waist circumference with other baseline biomarkers were tested using linear regression to confirm internal validity of the data.

The impacts of the intervention on biomarkers were explored by linear regression. The effect of the intervention on each variable was estimated by comparing the mean change from baseline in the intervention group with the corresponding mean change in the control group; this approach adjusted for baseline imbalances between arms. Variables for which the underlying distributions were skewed were transformed to a logarithmic scale before conducting any formal analysis. The results are presented as relative changes (with corresponding 95% CIs), either by exponentiation of the parameter estimates when data were analysed on a logarithmic scale or by expressing the estimated effect sizes as a percent of the overall mean baseline value for variables which were not transformed. The linearity and constant variance assumptions were checked by examining plots of residuals against fitted values.

We also investigated the effect of adiposity change on biomarkers using linear regression, utilising the whole cohort as a prospective cohort study regardless of intervention. Change in weight (per 1kg) and change in waist circumference (per 1cm) were modelled as explanatory

variables for changes in biomarkers. As above, skewed data were transformed to a logarithmic scale prior to analysis, and all estimates of effect size are expressed as percent change in the biomarker per 1kg change in weight or per 1cm change in waist circumference. We did not adjust for any factors in these analyses; randomised intervention is an effect mediator rather than a confounder, and demographic variables (age, sex, and ethnicity) are unlikely to be associated with both change in adiposity and change in biomarkers and hence meet the definition of confounders.

The trial met with limited success in recruiting family clusters [7]; in estimating the intra-class correlation coefficient (ICC) to account for clustering the relevant variance component was negative, so by convention, the estimated intra-class correlation was taken to be zero, and no adjustment for clustering was needed. The interpretation of the results requires context-dependant interpretation of the number of tests done, and acknowledgment of the possibility of chance findings rather than formal correction for multiple comparisons [14]. As such our pre-specified analyses were conducted and although not all models are presented here (such as scatter plots of distributions and linear regression models of baseline data), the data presented are consistent with those obtained in all analyses.

All data were analysed using SAS V 9.3 (SAS Institute Inc, Cary, North Carolina, USA).

RESULTS

Baseline characteristics

There were complete data available for 151 of the 171 randomised participants (88.3%) (Fig 1). Those with complete data had similar demographic characteristics compared to the complete trial group in terms of sex (44% vs. 46% men), age (mean 53 years vs. 52 years),

ethnicity (Indian 33% vs. 33%), weight (mean 103kg vs. 103kg), and randomised group (intervention 50% vs. 50%). Characteristics of the study by randomised group at baseline are described in Table 1. The demographic and anthropometric characteristics of the two groups were very similar, although the CRP, IL-6, insulin, HOMA-IR and triglyceride levels were slightly higher in the control group**.**

Correlations and associations at baseline

Baseline correlates of both weight and waist circumference (r>0.20) included HDL-cholesterol (inverse), CRP, leptin, insulin, HOMA-IR and diastolic blood pressure (all positive) (Supplemental Table S1).

Effect of intervention on biomarkers

Among those with complete data, mean weight loss in the intervention group was 1.14 kg, compared with a mean weight gain of 0.30 kg in the controls, an adjusted mean loss of 1.44 kg (95% CI 0.18 to 2.71) in the intervention group. Mean waist circumference reduction in the intervention group was 2.22cm, compared with a reduction of 0.63cm in the controls, an adjusted mean reduction of 1.59cm (0.08 to 3.09) in the intervention group. This translated into a 1.8% (95% CI 0.2 to 3.4) lower weight and a 1.5% (95% CI 0.1 to 3.0) lower waist circumference in the intervention group at follow-up (Table 2).

There was no evidence that the intervention had a significant effect on any mean values of the biomarkers of cardiometabolic risk (Table 2).

Effect of weight and waist circumference change on biomarkers using data from the entire cohort

In a linear regression model each 1kg weight reduction was associated with statistically significant relative changes in triglycerides (-1.3%, p=0.048) ALT (-2.5%, p=0.032), GGT (-2.2%, p=0.040), leptin (-6.5%, p<0.0001) insulin (-3.7% p=0.0005), fasting glucose (-0.8%, p=0.0071), 2-hour glucose (-2.3%, p=0.0002) HOMA-IR (-4.5%, p=0.0002) and absolute changes in these markers were commensurate (Table 3). The equivalent relative changes for 1cm smaller waist circumference were triglycerides (-1.1%, p=0.048) ALT (-2.2%, p=0.029), GGT (-1.2%, p=0.196), leptin (-4.0%, p=0.0005) insulin (-2.4% p=0.0105), fasting glucose (-0.6%, p=0.0217), 2-hour glucose (-1.7%, p=0.0010) HOMA-IR (-2.9%, p=0.0045) (Table 4). There was no evidence of an association of change in weight or waist circumference with t-PA, markers of inflammation (CRP and IL-6), or blood pressure (Table 3 and 4).

CONCLUSIONS

This study makes important observations regarding the effect of modest changes in adiposity on biomarkers of cardiometabolic risk in South Asians. Firstly, although the intervention resulted in an adjusted mean loss of 1.44kg and 1.59cm in waist circumference compared to controls over 3 years, in our RCT based analysis this did not lead to a change in biomarkers. This is likely due to the modest level of weight loss achieved and the considerable overlap in weight loss between the intervention and control group, and consequent lack of power in the randomised analysis. For example 23% of participants in the intervention arm and 19% of participants in the control gained more than 2.5kg [7]. Secondly, when analysed as a single cohort, the improved statistical power resulting from our exploiting the variability from participant to participant in weight change as a continuous variable enabled detection of associations of changes in weight and waist with some biomarkers i.e. in triglycerides, leptin, liver function tests (ALT and GGT) and glycaemia and insulin metabolism (insulin, fasting glucose, 2 hour glucose and HOMA-IR). The models suggest that changes in weight would

need to be more substantial for changes in biomarkers become clinically significant. There was, interestingly, no evidence of a relationship between change in weight and/or change in waist on inflammatory markers, CRP and IL-6, nor on blood pressure. The effects of weight loss on cardiometabolic biomarkers have been observed in studies of other ethnic groups [15–18], but not previously in South Asians. These data provide novel information on magnitude of the dose-response relationship between change in weight (and waist) and change in biomarkers in this ethnic group, although we must be cautious in interpretation of statistically significant small effects in the context of multiple testing and non-randomised data. Nevertheless, the pattern of results are internally consistent in that leptin is a strong biomarker of fat mass and ALT and GGT are documented surrogate markers of liver fat in those who do not drink alcohol excessively [19].

Non-alcoholic fatty liver disease (NAFLD) is an important clinical consequence of excess adiposity and can lead, in a minority of people, to more serious complications such as fibrosis and cirrhosis [19]. One of the important observations from our model is that reductions in weight were associated with reductions in ALT and GGT (>2% reduction per kg weight lost)**.** It is well-known that obesity, type 2 diabetes, and insulin resistance are closely linked to NAFLD [20] and lifestyle interventions such as weight loss and increased physical activity are associated with reductions in liver fat [21,22]. The lack of association between change in weight and waist circumference and markers of inflammation (CRP and IL-6) in the longitudinal data is therefore unexpected. This may simply be an issue of lack of power and is in keeping with data from the Finnish Diabetes Prevention Study in people with IGT [23].

Recent Mendelian randomisation analysis shows that the rs9939609 FTO single nucleotide polymorphism that is associated with small increases in BMI was associated with lower

HDL-cholesterol, higher insulin, higher 2-hour (but not fasting) glucose, higher liver enzymes (ALT and GGT), higher CRP (but not IL-6), higher triglycerides, and higher systolic and diastolic blood pressure [24]. The present study extends these findings by showing the effect of moderate weight loss in a prospective study comprising weight loss and physical activity elements which will not only impact adiposity, but also muscle mass. Like the Mendelian randomisation study, we show that the effect of modest weight loss on cardiometabolic biomarkers is small, and patients must be encouraged to prevent weight gain or to maximise weight loss in order to yield tangible health benefits from reducing cardiometabolic risk factors. It should also be noted that a Prevención con Dieta Mediterránea (PREDIMED) substudy suggested that diet quality (the Mediterranean diet) might beneficially influence cardiovascular risk factor biochemical parameters without specifically restricting calorie intake [25]. Although the PODOSA complex intervention encouraged healthy eating, the specific effect of this component of the intervention could not be investigated.

Strengths and weaknesses of the study require consideration. The first part of our study represents a randomised intervention trial, the gold standard for identifying causal mechanisms [26]. As discussed above, the small size of the study and the limited effect of the intervention on adiposity make power an issue for the study. Although use of prospective pooled data allows an analysis with greater power, we recognise that not all participants may have lost weight due to intentional weight change. That noted, their motivation for trial inclusion was to lose weight so it is likely that most lost weight intentionally. The pooled data ignores the effect of 3 years of ageing on biomarkers of interest, focusing on the effect of changing weight over that time. It is important to acknowledge that our study is based on modelling a linear relationship between weight change and subsequent changes in

cardiometabolic biomarkers. Extrapolating these models to situations where there is more pronounced weight loss may not be valid; for instance, there may be proportionally increased benefit from losing larger amounts of weight via threshold effects. Indeed, we know from studies in mainly White European origin subjects with diabetes that 8kg weight loss can substantially alter liver fat levels and can reverse diabetes [27]. How such weight loss can be achieved other than in highly motivated individuals is the topic of debate [28–30]. We were not able to assess the effect of more pronounced weight loss on biomarkers due to limited power. There is some potential that changes in medication during follow-up may impact both weight and biomarkers of risk, but we did not have sufficient data to sensibly adjust for such effects, which are expected to be small, and the randomised design of the trial should limit baseline imbalances.

In conclusion, the present study shows that a lifestyle intervention in South Asians with impaired fasting glucose or impaired glucose tolerance living in the UK which had only very moderate effects on adiposity did not improve biomarkers of cardiometabolic risk between study groups in a randomised comparison. Analysis as a single longitudinal cohort did, however, indicate that associations between adiposity changes and cardiometabolic biomarkers (specifically those related to total and ectopic fat mass and related glycaemia and insulin sensitivity) exist; decrease in weight was associated with favourable changes in markers in several domains of cardiometabolic risk.

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Conflict of Interest

JT received research grants, served as a consultant to and/or a member of advisory boards for, and/or gave lectures organized by AstraZeneca, Bayer, Boehringer Ingelheim, Eli Lilly, ImpetoMedical, Merck, MSD, Sanofi-Aventis, Novartis, Novo Nordisk and Servier. The remaining authors declare they have no conflicts of interest.

Supplementary information is available at IJO's website

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Figure Legends

Figure 1: Flow chart of participants who met inclusion/exclusion criteria for the study.

Table 1 Baseline characteristics of study participants by randomised group

	Characteristics at Baseline	
Characteristic	**Intervention group (n=75)**	**Control group (n=76)**
Clinical/Demographic		
Male sex	33 (44%)	34 (45%)
Age (years)	52.6 (10.3)	52.4 (9.8)
Location		
Glasgow	59 (79%)	59 (78%)
Edinburgh	16 (21%)	17 (22%)
Ethnic group		
Indian	26 (35%)	24 (32%)
Pakistani	49 (65%)	52 (68%)
Height (cm)	160.9 (10.6)	162.4 (8.0)
Anthropometric		
Weight (kg)	79.2 (16.7)	80.5 (15.5)
BMI (kg/m²)	30.5 (5.2)	30.5 (4.8)
Waist circumference (cm)	102.2 (11.3)	103.2 (11.4)
Lipids		
Total Cholesterol (mmol/L)	4.55 (0.84)	4.64 (0.95)
HDL-c (mmol/L)	1.08 (0.31)	1.04 (0.27)
Triglycerides (mmol/L)	1.50 (0.56)	1.64 (0.65)
LDL-C (mmol/L)	3.17 (0.81)	3.27 (0.94)
Liver function tests		
AST (U/L)	32.0 [25.8,39.8]	31.7 [22.8,40.1]
ALT (U/L)	22.9 [16.5,31.4]	23.8 [15.3,30.8]
GGT (U/L)	30 [19,41]	25 [19,41]
Inflammatory/endothelial		
t-PA (ng/ml)	14.07 (7.46)	13.35 (6.41)
CRP (mg/L)	2.82 [1.12,4.60]	3.45 [1.28,5.59]
IL-6 (pg/ml)	1.15 [0.76,2.09]	1.28 [0.83,2.14]
Metabolic		
Leptin (ng/ml)	27.2 [14.4,45.0]	33.9 [17.8,52.8]
Insulin (μU/L)	13.6 [8.5,17.1]	15.1 [10.7,19.4]
HOMA-IR	3.54 [2.24,4.58]	3.91 [2.59,5.12]
Fasting plasma glucose (mmol/L)	5.80 (0.62)	5.81 (0.61)
2 hour plasma glucose (mmol/L)	8.13 (1.65)	8.26 (1.52)
Blood pressure		
Systolic blood pressure (mmHg)	137.5 (22.8)	137.5 (19.5)
Diastolic blood pressure (mmHg)	82.3 (13.1)	83.91 (10.7)

Continuous data are presented as means and standard deviations or medians and interquartile ranges; categorical data presented as numbers and percentages.

Table 2 Relative change in active intervention versus control group (95% CI, p-values) in weight, waist circumference and biomarkers for the 151 participants with complete data

Characteristic	Absolute change			Effect estimate* (95% CI)	p-value
	Intervention group (3 year – baseline)	Control group (3 year – baseline)	Intervention vs Control (95% CI)		
Anthropometric					
Weight (kg)	-1.14 (4.22)	0.30 (3.62)	-1.44 (-2.71 to -0.18)	-1.8% (-3.4%, -0.2%)	0.0256
Waist circumference (cm)	-2.22 (4.68)	-0.63 (4.68)	-1.59 (-3.09 to -0.08)	-1.5% (-3.0%, -0.1%)	0.0390
Lipids					
Total Cholesterol (mmol/L)	0.13 (0.83)	0.10 (0.80)	0.03 (-0.23 to 0.30)	0.7% (-5.0%, 6.6%)	0.7947
HDL-c (mmol/L)	0.08 (0.20)	0.03 (0.14)	0.05 (0.00 to 0.11)	5.0% (-0.3%, 10.4%)	0.066
Triglycerides (mmol/L)	-0.04 (0.55)	0.08 (0.47)	-0.12 (-0.28 to 0.04)	-7.6% (-17.8%, 2.5%)	0.1501
LDL-C (Friedewald)	0.05 (0.78)	0.05 (0.71)	0.01 (-0.23 to 0.24)	0.2% (-7.2%, 7.5%)	0.9667
Liver function					
AST (µ/L)	1.90 [-9.10,12.10]	-0.85 [-9.95,9.00]	1.22 (-5.05 to 8.77)	3.4% (-14.2%, 24.6%)	0.7220
ALT (µ/L)	0.50 [-7.80,5.40]	-3.60 [-10.80,2.45]	4.16 (-0.92 to 10.26)	16.0% (-3.5%, 39.6%)	0.1137
GGT (µ/L)	0.00 [-9.00,3.00]	-1.00 [-8.50,4.50]	4.21 (-3.36 to 13.17)	9.5% (-7.5%, 29.6%)	0.2917
Inflammatory/endothelial					
t-PA (ng/ml)	0.94 (10.69)	0.50 (8.38)	0.45 (-2.64 to 3.53)	3.2% (-19.1%, 25.5%)	0.7765
CRP (mg/L)	0.07 [-1.15,1.10]	0.12 [-1.81,1.36]	0.80 (-0.48 to 2.50)	18.1% (-10.9%, 56.6%)	0.2447
IL-6 (pg/ml)	-0.24 [-0.87,0.08]	-0.24 [-0.81,0.28]	0.02 (-0.32 to 0.44)	1.3% (-17.3%, 24.1%)	0.8999
Metabolic					
Leptin (ng/ml)	-1.00 [-7.31,5.82]	2.57 [-2.93,9.69]	-5.13 (-11.48 to 2.78)	-13.7% (-30.8%, 7.5%)	0.1883
Insulin (µm/L)	1.13 [-3.71,3.86]	-0.07 [-4.09,4.20]	2.24 (-0.62 to 5.64)	14.3% (-4.0%, 36.1%)	0.1307
HOMA-IR	0.29 [-0.84,1.41]	-0.16 [-1.21,1.33]	0.58 (-0.23 to 1.57)	14.3% (-5.6%, 38.5%)	0.1694
Fasting plasma glucose (mmol/L)	0.08 (0.68)	0.10 (0.91)	-0.01 (-0.27 to 0.25)	-0.2% (-4.7%, 4.3%)	0.9187
2 hour plasma glucose (mmol/L)	-0.69 (2.68)	-0.30 (2.44)	-0.40 (-1.22 to 0.43)	-4.8% (-14.8%, 5.2%)	0.3447
Blood pressure					
Systolic blood pressure (mmHg)	0.14 (16.94)	1.19 (18.21)	-1.05 (-6.71 to 4.61)	-0.8% (-4.9%, 3.4%)	0.7141
Diastolic blood pressure (mmHg)	-1.07 (10.87)	-1.07 (10.84)	0.00 (-3.49 to 3.49)	0.0% (-4.2%, 4.2%)	0.9996

*Estimates are the relative difference between the intervention and control groups.
Absolute change data are presented as means and standard deviations or medians and interquartile ranges

Table 3 Association between change in weight and change in biomarker

Characteristic	Per 1kg reduction in weight		p-value
	Absolute Change (95% CI)	Relative Change (95% CI)	
Lipids			
Total Cholesterol (mmol/L)	-0.03 (-0.06 to 0.00)	-0.6% (-1.4% to 0.1%)	0.0787
HDL-c (mmol/L)	0.00 (-0.01 to 0.01)	0.2% (-0.5% to 0.9%)	0.5034
Triglycerides (mmol/L)	-0.02 (-0.04 to 0.00)	-1.3% (-2.6% to 0.0%)	0.0484
LDL-C (mmol/L)	-0.03 (-0.06 to 0.00)	-0.9% (-1.8% to 0.1%)	0.0697
Liver function			
AST (U/L)	-0.14 (-0.98 to 0.68)	-0.4% (-2.8% to 1.9%)	0.7246
ALT (U/L)	-0.64 (-1.21 to -0.05)	-2.5% (-4.7% to -0.2%)	0.0323
GGT (U/L)	-0.97 (-1.87 to -0.04)	-2.2% (-4.2% to -0.1%)	0.0397
Inflammatory/endothelial			
t-PA (ng/ml)	0.17 (-0.21 to 0.56)	1.3% (-1.5% to 4.0%)	0.3772
CRP (mg/L)	-0.06 (-0.21 to 0.10)	-1.4% (-4.8% to 2.2%)	0.4496
IL-6 (pg/ml)	0.01 (-0.03 to 0.06)	0.6% (-1.9% to 3.3%)	0.6412
Metabolic			
Leptin (ng/ml)	-2.42 (-3.32 to -1.54)	-6.5% (-8.9% to -4.1%)	<0.0001
Insulin (μU/L)	-0.58 (-0.91 to -0.26)	-3.7% (-5.8% to -1.7%)	0.0005
Fasting plasma glucose (mmol/L)	-0.04 (-0.08 to -0.01)	-0.8% (-1.3% to -0.2%)	0.0071
2 hour plasma glucose (mmol/L)	-0.19 (-0.29 to -0.09)	-2.3% (-3.5% to -1.1%)	0.0002
HOMA-IR	-0.18 (-0.27 to -0.09)	-4.5% (-6.7% to -2.2%)	0.0002
Blood pressure			
Systolic BP (mmHg)	-0.41 (-1.12 to 0.29)	-0.3% (-0.8% to 0.2%)	0.2506
Diastolic BP (mmHg)	-0.27 (-0.71 to 0.17)	-0.3% (-0.9% to 0.2%)	0.2229

Table 4 Association between change in waist circumference and change in biomarker

Characteristic	Per 1cm reduction in waist		p-value
	Absolute Change (95% CI)	Relative Change (95% CI)	
Lipids			
Total Cholesterol	-0.01 (-0.04 to 0.01)	-0.3% (-0.9% to 0.3%)	0.3459
HDL-c	0.00 (0.00 to 0.01)	0.2% (-0.4% to 0.8%)	0.4399
Triglycerides	-0.02 (-0.04 to 0.00)	-1.1% (-2.2% to 0.0%)	0.0483
LDL-C	-0.01 (-0.04 to 0.01)	-0.4% (-1.2% to 0.4%)	0.3445
Liver function			
AST	-0.28 (-0.98 to 0.43)	-0.8% (-2.8% to 1.2%)	0.4343
ALT	-0.56 (-1.04 to -0.05)	-2.2% (-4.0% to -0.2%)	0.0291
GGT	-0.53 (-1.31 to 0.27)	-1.2% (-3.0% to 0.6%)	0.1960
Inflammatory/endothelial			
t-PA	0.15 (-0.17 to 0.48)	1.1% (-1.2% to 3.5%)	0.3487
CRP	-0.06 (-0.19 to 0.07)	-1.4% (-4.3% to 1.6%)	0.3544
IL-6	-0.01 (-0.05 to 0.03)	-0.4% (-2.5% to 1.8%)	0.7231
Metabolic			
Leptin	-1.50 (-2.28 to -0.67)	-4.0% (-6.1% to -1.8%)	0.0005
Insulin	-0.37 (-0.64 to -0.09)	-2.4% (-4.1% to -0.6%)	0.0105
Fasting glucose	-0.03 (-0.06 to -0.01)	-0.6% (-1.0% to -0.1%)	0.0217
2-hr glucose	-0.14 (-0.23 to -0.06)	-1.7% (-2.8% to -0.7%)	0.0010
HOMA-IR	-0.12 (-0.19 to -0.04)	-2.9% (-4.8% to -0.9%)	0.0045
Blood pressure			
Systolic BP	-0.27 (-0.87 to 0.33)	-0.2% (-0.6% to 0.2%)	0.3747
Diastolic BP	-0.29 (-0.66 to 0.08)	-0.3% (-0.8% to 0.1%)	0.1223