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ORIGINAL ARTICLE

Effect of lifestyle improvement program on the biomarkers of adiposity, inflammation and gut hormones in overweight/obese Asian Indians with prediabetes

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Abstract
Aims While lifestyle modification is known to offer several metabolic benefits, there is paucity of comprehensive data on changes in biomarkers of adiposity, inflammation as well as gut hormones. We investigated these biomarkers in overweight/obese individuals with prediabetes randomized to either 4 months of a lifestyle improvement program or standard care and followed them up for a year.
Methods Participants [standard care and intervention arm ($n = 75$ each)] were randomly selected from the Diabetes Community Lifestyle Improvement Program trial. Glycemic and lipid control and anthropometric measurements were assessed by standard protocols. Adipokines, inflammatory markers and gut hormones were measured using multiplex and standard ELISA kits.
Results Along with modest benefits in primary outcomes (glycemic and lipid control and weight reduction), participants in the intervention group showed significant reductions ($p < 0.001$) in plasma levels of leptin (17.6%), TNF-α (35%), IL-6 (33.3%), MCP-1 (22.3%) and PYY (28.3%) and increased levels of adiponectin (33.1%) and ghrelin (23.6%) at the end of 4 months of lifestyle intervention. The changes were independent of weight and persisted even at 1 year of follow-up. In contrast, participants from the standard care arm did not show any statistically significant improvements on the above parameters.
Conclusions Participants who underwent an intensive lifestyle improvement program showed metabolic benefits as well as favorable beneficial changes in systemic levels of adipokines, cytokines and gut hormones, not only during the intervention period, but also during 12-month follow-up period.

Keywords Lifestyle intervention · Adiposity · Inflammation · Gut hormones · Obesity and Asian Indians

Introduction

Obesity is a well-known contributor to the risk of type 2 diabetes mellitus (T2DM) and other cardiometabolic conditions and also believed to play a key role in chronic inflammation and insulin sensitivity [1]. Observational and randomized clinical trials have demonstrated the benefits of regular physical activity and healthy behaviors in maintaining metabolic control and reducing the incidence of T2DM and associated risk factors such as obesity among populations at risk of T2DM (e.g., those with prediabetes) [2]. These interventions have also been shown to improve markers of glucose tolerance and insulin sensitivity, blood pressure and plasma lipid levels [3, 4]. A recent study by Magkos et al. [5] reported that a 5% weight loss improves metabolic function in multiple organs simultaneously and progressive weight loss causes dose-dependent alterations

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in adipose tissue and beta cell function. The results of lifestyle interventions on adiposity, metabolism, inflammation and physical fitness immediately after treatment are promising. However, the long-term follow-up effects of such interventions on adiposity, inflammation and gut hormones along with glycemic and lipid control are less studied.

Asian Indians are especially susceptible to cardiometabolic diseases [6, 7] and report low levels of physical activity [8] that predispose them to developing cardiometabolic defects in general and T2DM in particular. Our earlier studies demonstrated that the levels of inflammatory markers increase with increasing severity of glucose intolerance [9, 10]. Extending our systemic level observation on inflammatory markers, we also studied the peripheral blood mononuclear cells (PBMCs) and demonstrated increased proinflammatory and prooxidant gene expression patterns not only in patients with T2DM but also in individuals with prediabetes [11]. Thus, subjects with prediabetes/obesity exhibit significant alterations in systemic levels of certain specific adipokines, cytokines and gut hormones. However, there is lack of data on the effect of lifestyle intervention in relation to biomarkers of adiposity, inflammation and gut hormones in Asian Indians. We therefore comprehensively investigated the changes in the biomarkers of adiposity (adiponectin, leptin), inflammation [tumor necrosis factor alpha (TNF-α), interleukin-6 (IL-6), monocyte chemotactic protein (MCP-1)] and gut hormones [ghrelin, Peptide YY (PYY)] in participants of the Diabetes Community Lifestyle Improvement Program (D-CLIP) trial, a diabetes prevention program [12, 13] conducted in overweight/obese adults with prediabetes.

Materials and methods

The Diabetes Community Lifestyle Improvement Program (D-CLIP) is a randomized, controlled, translation trial of 578 overweight/obese Asian Indian adults aged 20–65 years with isolated impaired glucose tolerance (iIGT), isolated impaired fasting glucose (iIFG), or IFG + IGT in Chennai, India [12]. Study participants were randomized to receive either the intervention or standard lifestyle advice (control arm) over a period of 3 years. Study methods have been described elsewhere [12, 13]; however, the study protocol pertinent to this paper has been detailed here. The step-up model for diabetes prevention included 16 weeks of intensive lifestyle intervention (diet and exercise) modeled after the US Diabetes Prevention Program (DPP) [4]. Control arm participants received the study site's standard of care for prediabetes: a single day one-on-one visits with a physician, a dietitian and a fitness trainer and one common group education class on diabetes prevention (e.g., following a low-fat diet rich in complex carbohydrates and fresh fruits and vegetables and increasing physical activity). Control and intervention activities were conducted at the study site, a diabetes care and research institution in Chennai, India, with extensive experience in diabetes treatment and prevention. At the end of 16 weeks (4 months) of structured lifestyle intervention classes, blood samples were analyzed for metabolic parameters. Individuals who were identified to have IFG + IGT at this stage were determined to be at the highest risk to develop diabetes and prescribed the metformin (500 mg twice a day) as a step-up therapy. Both standard care and intervention arm study participants were not on any anti-inflammatory medication or any other medications. None of the standard care arm study participants were on metformin treatment.

For this sub-study of D-CLIP, a total of 150 participants (75 from the intervention arm and 75 from the control arm) were randomly selected from D-CLIP cohort with follow-up data at 4 and 12 months. Biochemical assays were measured at three time points; baseline, at 4 months post-active lifestyle intervention and at 12-month follow-up (Supplementary Figure 1). Sample size was calculated to have at least 80% power for obtaining a correlation coefficient of 0.3 between the study parameters, with an alpha error of 0.01 in each study group. From our previous study on adiponectin [14], we calculated that '*n*' size of 60 had a power of >80% to detect a difference of 2 μg/ml between groups for adiponectin, with a standard deviation of 0.5 and an alpha error of 0.05. The sample size was based on the primary outcome of 2 μg/ml between groups for adiponectin and lesser for other biomarkers.

All study materials received approvals from the Emory University Institutional Review Board and the Madras Diabetes Research Foundation Ethics Committee (Clinicaltrials.gov NCT01283308). Weight, height and waist circumference were obtained by trained data collectors using standardized methods [12]. BMI was calculated as weight (kg) divided by height (m) squared. Blood pressure was recorded from the right arm in a sitting position to the nearest 2 mmHg with a mercury sphygmomanometer (Diamond Deluxe BP apparatus, Pune, India). Two readings were taken 5 min apart, and the mean of the two was taken as the blood pressure. Fasting plasma glucose (hexokinase method), serum cholesterol (cholesterol oxidase–peroxidase–amidopyrine method), serum triglycerides (glycerol phosphate oxidase–peroxidase–amidopyrine method) and HDL cholesterol (direct method–polyethylene glycol–pretreated enzymes) were measured using Hitachi-912 Autoanalyzer (Hitachi, Mannheim, Germany). Low-density lipoprotein (LDL) cholesterol was calculated using the Friedewald formula. Glycated hemoglobin (HbA1c)

was measured by high-pressure liquid chromatography using the Variant machine (Bio-Rad, Hercules, California, USA). Serum insulin concentration was estimated using the electrochemiluminescence method (COBAS E 411; Roche Diagnostics). The intra- and inter-assay coefficients of variation for the biochemical assays ranged between 3.1 and 7.6%. All measurements were performed in the laboratory at the study site (the Madras Diabetes Research Foundation, Chennai, India), which is certified by the College of American Pathologists (Northfield, IL) (No. 7214031) and the National Accreditation Board for Testing and Calibration of Laboratories (New Delhi, India) (M0226).

The following assays were performed: adipokines (adiponectin, leptin), inflammatory cytokines (TNF-α, IL-6 & MCP-1) and gut hormones (ghrelin and PYY) at three different points of the D-CLIP study (Supplementary Figure 1), viz. baseline (at 0 months), end of active lifestyle intervention (at the 4th month after randomization) and follow-up (at the 12th month after randomization). Adipokines and inflammatory cytokines were measured by multiplex enzyme-linked immunosorbent assay (ELISA) with the use of the human adipokine/cytokine panel in the same assay (LINCOplex Kit, Millipore). This multiplex assay kit uses antibody-immobilized beads to simultaneously quantify these peptide hormones [15]. Intra-assay and inter-assay variations were <10 and <18%, respectively.

Ghrelin and PYY were measured by ELISA (USCN, USA). In brief, monoclonal antibody specific to ghrelin and PYY were precoated onto a microplate. Standards and samples were pipetted into the wells so that any ghrelin and PYY present will be bound to the immobilized antibody. After washing away any unbound substances, an enzyme-linked polyclonal antibody specific for ghrelin and PYY was added to the wells. Following this, a substrate solution was added for color development (blue to yellow) in proportion to the amount of ghrelin and PYY concentration in the sample. The color development was stopped with the stop solution, and the intensity of the color was read at 450 nm. The values were expressed in pg/ml units. The intra- and inter-assay coefficients of variation were <5 and <10%, respectively.

Normal glucose tolerance (NGT) was defined as fasting plasma glucose <5.6 mmol/l (<100 mg/dl) and 2-h post-glucose value <7.8 mmol/l (<140 mg/dl) as NGT [16, 17]. Prediabetes included IFG and IGT. Those with fasting plasma glucose <5.6 mmol/l (<100 mg/dl) and 2-h post-glucose value ≥7.8 mmol/l (≥140 mg/dl) and <11.1 mmol/l (<200 mg/dl) were diagnosed as IGT [16, 17]. IFG was defined as fasting plasma glucose values between 100 and 125 mg/dl (5.6–6.9 mmol/l), with 2-h post-glucose <140 mg/dl (7.8 mmol/l) [16, 17]. The World Health Organization (WHO) Asia Pacific BMI cut point of <25.0 or ≥25.0 kg/m^2 was used to define obesity [18]. Insulin resistance was defined using the homeostasis model assessment–insulin resistance (HOMA-IR) formula: HOMA-IR = fasting insulin (μIU/ml) * fasting glucose (mmol/l)/22.5 [19].

Statistical analysis

Data were analyzed using SPSS statistical package (version 15.0, Chicago, IL). Continuous variables were tested for normality, and non-normal values were transformed. Unadjusted comparisons between study arms were made using *t* tests or Chi-square tests. Using repeated measure analysis under General Linear Model (GLM), we estimated the mean difference within groups (across 3 times points within a group) as well as across groups over time (intervention/control). The within-subject variables include the hormone/biomarker at each time point and between-subject factor was the arm (intervention or control). The post hoc test used to analyze the differences across three time points was the LSD (Least Significant Difference). As per D-CLIP protocol (already detailed in the methodology), at the end of 4 months of intervention, participants at the highest risk to develop diabetes were prescribed metformin. For this sub-study, of the 75 intervention group participants, 30 were identified to be at high risk at the end of 4 months of intervention (40%). A sensitivity analysis was carried out to study the effect of metformin on the results of this study.

Results

At baseline, participant's characteristics in the standard care as well as intervention group were similar (Table 1). The mean age of the participants was 44.5 years, mean BMI 27.6 kg/m^2, and mean body weight 74.6 kgs. Out of a total of 150 subjects included in the study, 60.6% ($n = 91$) were male.

Compared to standard care arm, participants in the intervention group experienced greater improvements in body weight, waist circumference, systolic and diastolic blood pressure ($p < 0.05$ for each comparison) fasting plasma glucose ($p < 0.05$), LDL cholesterol ($p < 0.05$) and HOMA-IR ($p < 0.05$) at the end of 4-month intervention. In the standard care group, there were no significant changes in body weight, waist circumference, HOMA-IR, glucose and lipid homeostasis (Table 1).

Effect of intervention on adiposity, inflammation and gut hormones

a. Changes in adipokines and inflammatory markers

At the end of the 4-month intervention, participants in the intervention arm showed a greater relative reduction in

Table 1 Clinical characteristics of study participants in standard care and intervention arm

Parameters	Standard care arm ($n = 75$)			Intervention arm ($n = 75$)		
	Baseline	4th month	12th month	Baseline	End of 4-month intervention	12-month follow-up
Body weight (kg)[#]	74.6 ± 10.6	73.6 ± 10.8	73.8 ± 11.5	73.9 ± 10.3	69.0 ± 9.6*	70.3 ± 10.6*
Body fat (%)	29.6 ± 7.1	30.7 ± 7.9	30.6 ± 7.7	29.1 ± 8.3	28.2 ± 8.1	29.3 ± 8.7
Waist circumference (cm)[#]	94.5 ± 9.0	92.5 ± 9.1	92.3 ± 8.9	93.5 ± 9.2	89.4 ± 9.3*	89.7 ± 9.1*
Body mass index (kg/m^2)[#]	28.5 ± 4.6	28.2 ± 4.5	28.1 ± 4.5	27.5 ± 3.9	26.5 ± 3.8	26.6 ± 3.9
Systolic blood pressure (mm Hg)[#]	124.6 ± 14.6	121.1 ± 12.0	122.8 ± 13.7	122.0 ± 12.3	116.0 ± 12.4*	119.0 ± 10.8*
Diastolic blood pressure (mm Hg)[#]	74.3 ± 10	72.6 ± 9.3	73.3 ± 10	74.0 ± 8.8	70.7 ± 8.8*	69.8 ± 8.6*
HOMA-IR[#]	3.5 ± 1.7	3.4 ± 1.6	3.2 ± 1.7	3.3 ± 1.8	2.2 ± 1.3*	2.5 ± 1.5*
Fasting plasma glucose (mg/dl)[#]	104.5 ± 9.3	101.5 ± 9.1	102.0 ± 9.3	104.4 ± 8.9	99.6 ± 8.3*	98.5 ± 9.4*
Total serum cholesterol (mg/dl)	180 ± 32	177 ± 31	178 ± 32	183 ± 32	172 ± 26*	174 ± 27
Serum triglycerides (mg/dl)	140 ± 55	138 ± 54	139 ± 56	142 ± 46	128 ± 39*	135 ± 40*
Serum HDL cholesterol (mg/dl)	39.4 ± 6.5	39.7 ± 5.8	39.9 ± 7.0	39.2 ± 7.2	38.5 ± 6.7	41.9 ± 7.6
Serum LDL cholesterol (mg/dl)[#]	114 ± 30	113 ± 28	112 ± 29	111 ± 26	102 ± 23*	105 ± 25

* $p < 0.05$ compared to baseline values in intervention arm

[#] $p < 0.05$ (overall across the three time points) when comparing intervention arm to control arm using generalized linear model (repeated measures analyses)

leptin (107 pg/ml; 9.5%), TNF-α (7.9 pg/ml; 24.3%), MCP-1 (74 ng/ml; 14%) and IL-6 (72 pg/ml; 24.9%) ($p < 0.001$ for each comparison) and greater increase in levels of adiponectin (40 ng/ml; 23.3%, $p < 0.001$) compared to standard care arm.

Compared to the baseline values, at 4 months, participants who received lifestyle improvement program showed significant reductions in plasma levels of leptin (197 pg/ml; 17.6%), TNF-α (11.4 pg/ml; 35%), MCP-1 (119 ng/ml; 22.3%) and IL-6 (99 pg/ml; 33.3%) ($p < 0.001$ for each comparison) and higher levels of adiponectin (74 ng/ml; 33.1%) ($p < 0.001$). At 1-year follow-up, there was persistent decrease in the mean levels of leptin (171 pg/ml; 15.3%), TNF-α (8.5 pg/ml; 26%), MCP-1 (104 ng/ml; 19.5%), IL-6 (84 pg/ml; 29.2%) ($p < 0.001$ for each), and increase in adiponectin levels (62 ng/ml; 28.2%) ($p < 0.001$) in the intervention arm participants compared to baseline. The standard care arm showed no significant differences in adipokines or cytokines at the end of 4-month intervention or at 1 year, compared to baseline (Figs. 1a–c, 2a, b).

b. Changes in gut hormones:

Compared to the standard care group, intervention arm participants showed a greater reduction in PYY levels (2.9 pg/ml; 20.1%) and improvement in ghrelin levels (30 pg/ml; 15.2%) (p for each <0.001) at the end of 4-month intervention and the same trend was seen till the end of 12-month follow-up.

Compared to baseline values, participants in the intervention group showed a decrease in mean levels of PYY by 28.3% (4 pg/ml; $p < 0.001$) after the 4 months of lifestyle improvement program and still remained lower (3.6 pg/ml; 25.6%) at the end of 12-month follow-up ($p < 0.001$). Ghrelin levels were significantly increased in the intervention arm participants at 4 months post-intervention (46.3 pg/ml; 23.6%, $p < 0.001$) compared to baseline; they decreased marginally from 4 months to 1 year, but they remained significantly higher (35.2 pg/ml; 17.9%) ($p < 0.001$) than baseline values.

In the standard care arm, there were no significant changes in the levels of gut hormones at 4 months or 1 year (Fig. 3a, b) compared to baseline values.

c. Metformin treatment:

In the cohort of 75 intervention group participants, 30 were identified to be at high risk at the end of 4 months of intervention (40%). As the metformin treatment [20, 21] could confound the results at the end of 12 months, a sensitivity analysis was carried out at the follow-up stage comparing the intervention cohort with and without metformin. There were no statistically significant differences in adipokines, inflammatory markers or gut hormone levels in those with and without metformin treatment [Supplementary Figures (2-4)].

d. Effect of weight change on hormones:

Compared to standard care arm, participants in the intervention group experienced significant weight reduction ($p < 0.05$) at the end of 4-month intervention and remained consistently lower than the baseline values even at the end of the 12-month follow-up. Mixed methods

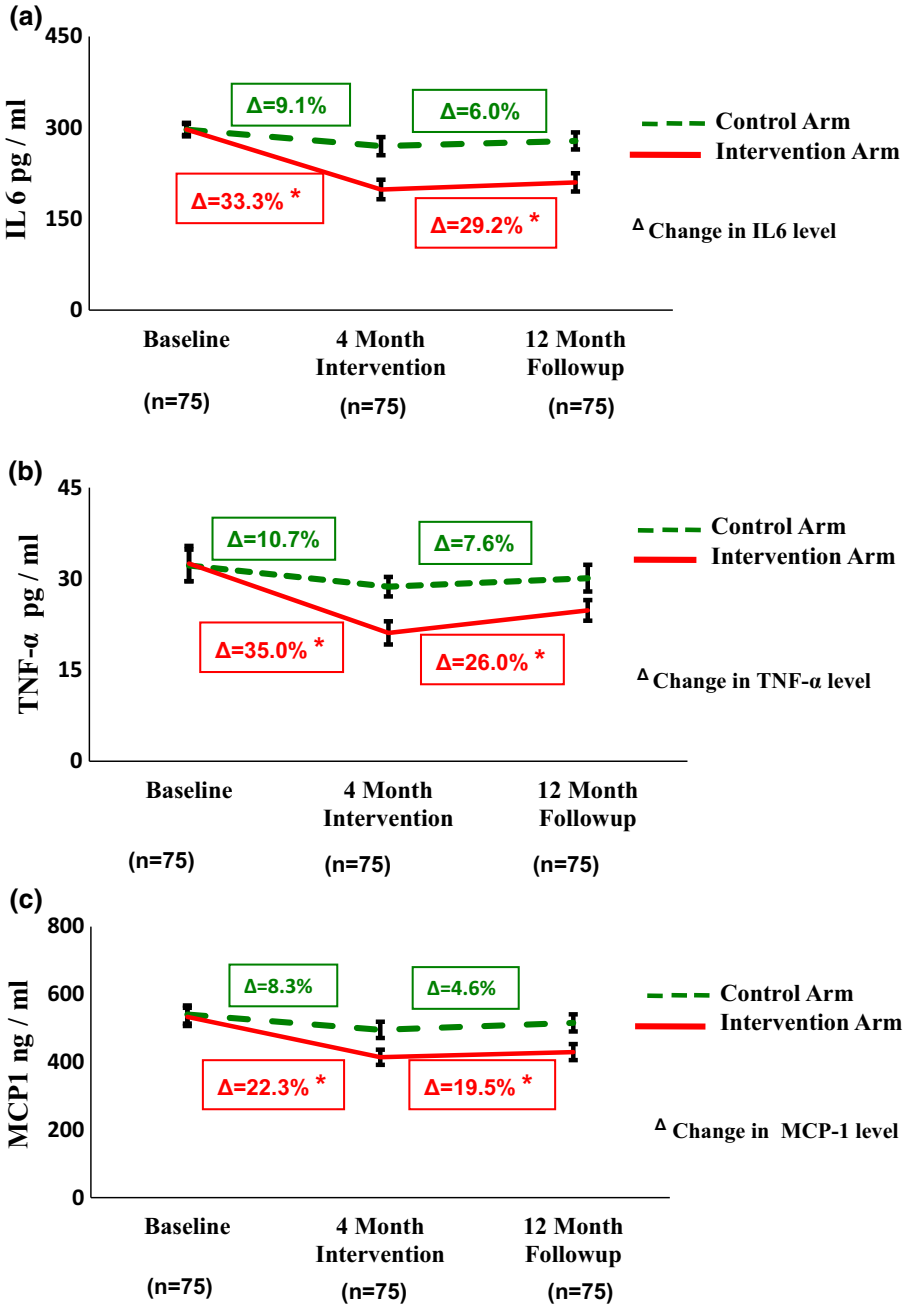

Fig. 1 Mean levels of inflammatory markers in relation to lifestyle intervention in DCLIP study. **a** Mean IL-6 levels, $*p < 0.001$ compared to baseline in the intervention arm. Data presented as mean (±SEM). **b** Mean TNF-α levels, $*p < 0.001$ compared to baseline in the intervention arm. Data presented as mean (±SEM). **c** Mean MCP-1 levels, $*p < 0.001$ compared to baseline in the intervention arm. Data presented as mean (±SEM)

analysis was done using the GLM mix function to examine whether the change in weight mediated the changes in the inflammatory markers, adipokines and gut hormones. It was found that changes in inflammatory markers, adipokines and gut hormones were significant even after adjusting for weight changes at the end of 4-month intervention and remained consistently significant even at the end of the 12-month follow-up [Supplementary Table 1]. After adjusted for fasting glucose and insulin resistance in the analysis, PYY only showed statistical difference at baseline between the two arms.

Discussion

Landmark diabetes prevention studies such as the US Diabetes Prevention Program, the Da Qing study, the Finnish Diabetes Prevention Study and the Indian Diabetes Prevention Program have shown that lifestyle intervention or metformin can reduce the incidence of T2DM in the range of 30–60% among individuals with IGT [3, 4, 22, 23]. The D-CLIP is the only diabetes prevention trial to our knowledge that has targeted individuals across the full prediabetes spectrum (i.e., IFG, IGT and

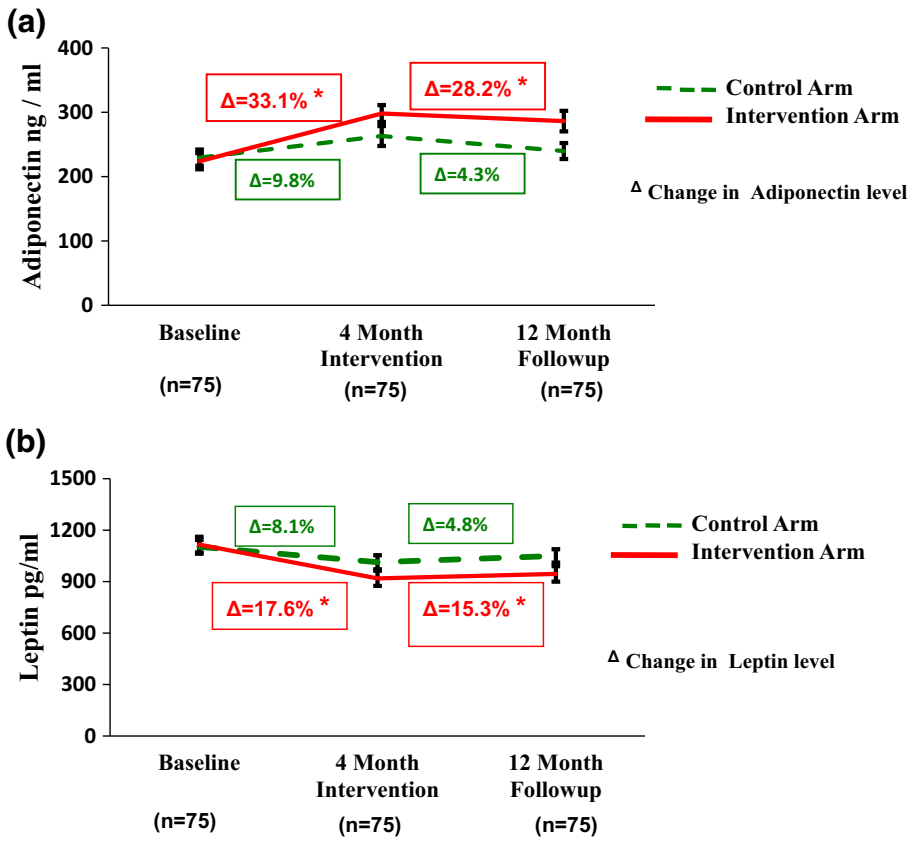

Fig. 2 Mean levels of adipokines in relation to lifestyle intervention in DCLIP study. **a** Mean adiponectin levels, $*p < 0.001$ compared to baseline in the intervention arm. Data presented as mean (±SEM). **b** Mean leptin levels, $*p < 0.001$ compared to baseline in the intervention arm. Data presented as mean (±SEM)

IFG + IGT), and tested the effectiveness of the stepwise diabetes prevention recommendations of lifestyle intervention [13]. The present study, which is a sub-study of the D-CLIP trial, assumes significance for the following: Our study reports that in participants who underwent an intensive lifestyle improvement program, the glycemic and lipid control and weight reduction were in parallel beneficially accompanied by robust changes in systemic levels of adipokines, cytokines and gut hormones, not only during the intervention period, but these changes persisted for 1 year post-intervention. In contrast, participants from the standard care arm did not show any statistically significant improvements on the above parameters. This implies that a single visit consultation with a dietitian, physical trainer and physician may not influence the participants in the standard care arm to such an extent that they would make sustainable changes in their overall lifestyle.

The effects of lifestyle modification on weight reduction and prevention of T2DM are well known [3, 4, 22]. However, the effects of these interventions on adipokines, inflammatory markers or gut hormones are less studied, especially in non-European populations. Asian Indians are known to be more insulin-resistant, have higher rates of T2DM at younger ages [24] and show earlier impairments in β-cell function [25]. In addition, serum adiponectin [26] levels are lower among Asian Indians, and the levels of inflammatory markers such as high sensitivity C-reactive protein (hsCRP) are higher [27]. These changes predispose them to cardiometabolic disorders in general and T2DM in particular. In this context, a sustained decrease in systemic levels of inflammation seen in our study with lifestyle intervention is an important observation.

There is cross-sectional evidence that lifestyle intervention is associated with lower systemic inflammation, but this has not been seen uniformly in all studies [28]. Data from small-scale intervention studies suggest that exercise training diminishes inflammation [29]. However, results from large, randomized, controlled trials (RCTs) designed to definitively test the effects of greater physical activity on inflammation were of inconclusive nature [30, 31]. Studies have reported that IL-6 levels increase with adiposity, and that 30% of circulating IL-6 might be released by adipose tissue in vivo [32]. Recently, soluble form of the activated leukocyte cell adhesion molecule (sALCAM), a potential biomarker of the innate immune system in inflammation-associated disorders, was shown to be significantly decreased after a lifestyle program [33]. In agreement with previous studies [34–36], our results showed significantly decreased levels of inflammatory markers (TNF-α, IL-6 and MCP-1) in participants under intervention compared to the standard care arm.

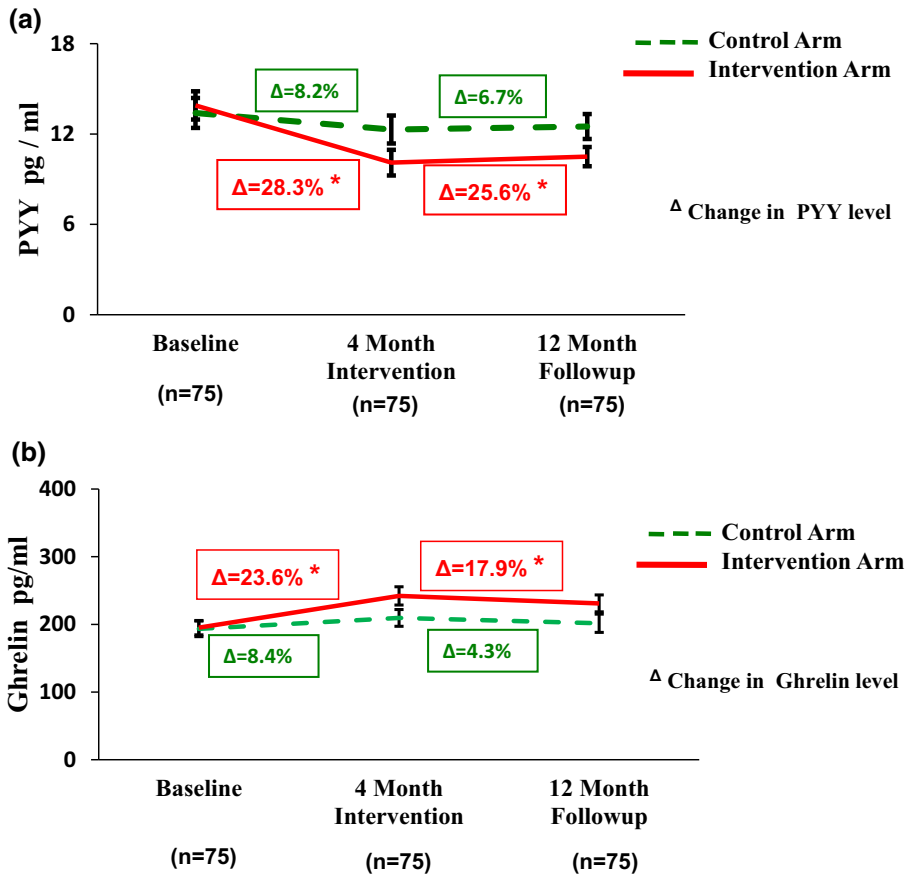

Fig. 3 Mean levels of gut hormones in relation to lifestyle intervention in DCLIP study. **a** Mean PYY levels, $*p < 0.001$ compared to baseline in the intervention arm. Data presented as mean (±SEM). **b** Mean ghrelin levels, $*p < 0.001$ compared to baseline in the intervention arm. Data presented as mean (±SEM)

Our study also showed beneficial alterations of adipokines (increased adiponectin and decreased leptin) in participants from the intervention arm. Weight reduction has been shown to increase serum adiponectin levels with beneficial effects on lipid metabolism and insulin sensitivity [37]. Hotta et al. [38] showed a significant increase in adiponectin levels in both diabetic patients and non-diabetic individuals after prolonged diet induced weight loss. Decreased serum levels of chemerin (adipokine) was also shown to be associated with improved insulin sensitivity after lifestyle intervention in overweight and obese type 2 diabetes patients [39]. In overweight and obese adults with type 2 diabetes, weight loss of 7–10% from a 1-year intensive lifestyle intervention resulted in significant reductions in all depots of adipose tissue including subcutaneous and visceral adipose compartments with improvements in metabolic indicators [40]. These results suggest that lifestyle modification through combined diet and exercise, in addition to improving insulin sensitivity and glucose homeostasis in obese individuals, also leads to favorable changes in adiposity determinants and systemic levels of adiponectin and leptin during the lifestyle intervention as well as post-intervention.

Glucose and insulin metabolism appears to be involved in the regulation of ghrelin levels due to the inverse relationships between this satiety factor and indices of insulin resistance [41]. PYY is a gut peptide produced in response to food intake and provides a satiety signal to the brain to terminate eating [42]. A prolonged caloric restriction has been showed to result in increases in ghrelin which may explain why bodyweight often stabilizes, despite continued attempts by individuals to adhere to prescribed weight loss diets [36, 43]. Ghrelin and PYY levels showed significant and beneficial changes in response to lifestyle intervention that persisted for 12 months in the current study. These results emphasize that a multitude of hormones or peptides are involved in the homeostatic regulation of body weight, many of which are altered positively even with marginal weight loss. Whether these changes represent a transient compensatory response to weight reduction is unknown, but an important finding of this study is that many of these beneficial alterations persist at 12 months post-intervention, suggesting a legacy effect of the lifestyle modification. Further studies are needed to identify newer biomarkers which could lead to better understanding of the underlying mechanisms in the different metabolic profile in Asian Indians.

Metformin therapy continued to show benefits during DPPOS follow-up [44] and thus further support the early adoption of metformin therapy in glucose intolerant individuals. Moreover, the American Diabetes Association now

recommends intensive lifestyle intervention or metformin to prevent diabetes for people at high risk, i.e., those with combined IFG + IGT [45]. Studies have reported that lifestyle modification alone or combined with metformin produced changes in adiponectin or other inflammatory markers [20], while others [21] have not found any correlation between metformin and adiponectin/inflammatory markers. In this study, we found that there were no statistically significant differences in adipokines, inflammatory markers or gut hormone levels in the intervention group with and without metformin treatment. This suggests that the effect of metformin in improving glucose control may be unrelated to the pathways of inflammatory, adiposity or gut hormones markers. However, the small number of participants in this study has limitation to make such claim, and hence, this should be investigated further in larger studies.

Given the heterogeneity of T2DM etiology and pathogenesis, it may be difficult to optimize global strategies for its prevention. The response to preventive interventions may differ greatly from one person to the other and in different ethnic groups. Although some of this variability could be attributed to varying levels of adherence to therapy and methodological factors, it may also be due to inter-individual biological differences in the way interventions work. Studies have demonstrated that lifestyle intervention transformed a deleterious insulin-resistant/proinflammatory profile into a more favorable profile with improved insulin action, endothelial function and reduced inflammation [46]. While abdominal obesity is a risk factor for T2DM and cardiometabolic disorders, it is also linked to changes in subclinical biomarkers at both the systemic level and tissue level (particularly adipose and enteroendocrine system). Furthermore, studies have reported that body mass index may not be sensitive marker for obesity-related risk of metabolic disorders [47, 48]. Recent studies have shown that a subset of obese individuals could also be metabolically healthy [49]. In this context, our study reporting the beneficial alterations of inflammatory markers, adipokines and gut hormones independently of weight change on lifestyle intervention is an important observation. Since many of the biomarkers studied in this study are also connected to epigenetic regulation, it is possible that these alterations could influence the differential responses of lifestyle in preventing diabetes across individuals. These data underscore the need for identifying new markers/biomarkers to disease prevention, based on accurate prognostic phenotyping of high-risk subjects and accurate selection of these individuals for the appropriately matched interventions. Furthermore, the Tubingen Lifestyle Intervention Program (TULIP) [50] emphasized that stratification of individuals with prediabetes at baseline into a high-risk and a low-risk phenotype would help to determine the effectiveness of a lifestyle intervention to revert individuals to normal glucose regulation, indicating that hormonal and other factors may play a role in determining the response to lifestyle modifications.

The strengths of the current study include measurement of multiple biomarkers at three time points which include before and after the intervention period from a randomized control trial—DCLIP. Secondly, prediabetes and overweight/obesity were defined and classified using standard methods, and individuals with both IFG and IGT were included. One of the limitations of our study is that due to logistic constraints, we studied the biomarkers only for a relatively shorter intervention and follow-up periods. Long-term studies are needed to more firmly establish the legacy effect on these biomarkers.

To conclude, this study is the first to our knowledge to comprehensively show the impact of a lifestyle intervention on favorable changes in adipokines, cytokines and gut hormones in overweight/obese Asian Indians with prediabetes. Interestingly these subclinical biomarkers were beneficially altered not only during the intervention period, but there appears to be a legacy effect on these measures even after the intervention is stopped, i.e., up to 12-month follow-up. Longer-term studies are needed to see how long these beneficial effects last.

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Compliance with ethical standards

Conflict of interest All authors have no relevant conflict of interest to disclose.

Ethical standard All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards.

Informed consent Informed consent was obtained from all patients for being included in the study which has done according to the ethical standards and in keeping with Helsinki Declaration of 2008 (ICH GCP).

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