Effect of weight loss on adipokine levels in obese patients

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Abstract

Background: Adipose tissue functions as an endocrine organ by releasing adipokines which have important roles in the regulation of inflammation and insulin sensitivity. Although there is evidence of improvements in the circulating levels of adipokines with weight loss, few studies relate such changes to specific diets. We investigated the effect of weight loss achieved by two different diets on circulating adipokine levels in obese individuals.

Methods: 120 obese patients (BMI ≥ 35 kg/m^2) underwent a screening period of 3-months on a low fat, reduced-calorie diet. Patients failing to achieve a 5% weight loss using this approach were randomly allocated to either a low carbohydrate/high protein diet (LCHP) (n=17) or a commercial very-low-calorie diet (LighterLife – LL) (n=14) for a period of 9-months.

Results: A significant weight loss (Kg) only maintained for LL at 9-months (-32.3 ± 22.7, $p < 0.0001$) but not LCHP. Changes in adiponectin (ng/ml) (15.8 ± 17.1 vs -0.8 ± 6.2, $p = 0.003$) and leptin (ng/ml) (-17.6 ± 24.3 vs -3.0 ± 9.2, $p = 0.049$) at 9-months were significantly greater for LL than for LCHP which may reflect greater weight loss and decrease in fat mass. Changes in TNF-α, IL-6 and PAI-1 did not differ significantly between the dietary interventions at 9-months.

Conclusions: A significant weight loss of 23.8% of baseline weight was observed using a very-low-calorie diet and resulted in significant improvements in circulating levels of leptin, PAI-1 and adiponectin, which are likely to be due to the weight loss and not the macronutrient intake. (ISRCTN: ISRCTN09760867)

Abbreviations: TNF, tumour necrosis factor; PAI-1, plasminogen activator inhibitor-1; IL-6, interleukin-6; RCT, randomised controlled trial; CDD, 600 calorie deficient diet; LCHP, low carbohydrate high protein; VLCD, very-low-calorie diet; LL, LighterLife; HDL, high density lipoprotein cholesterol; HOMA-IR, homeostasis model of assessment of insulin resistance; ELISA, enzyme-linked immunoassay.

Introduction

It is now evident that adipose tissue not only stores excess triacylglycerols, but functions as an endocrine organ by releasing adipokines, which have important roles in the in the regulation of appetite, glucose and lipid metabolism, inflammation, and insulin resistance [1,2]. Such adipokines include adiponectin, leptin, resistin, tumour necrosis factor (TNF)-α, plasminogen activator inhibitor-1 (PAI-1) and interleukin-6 (IL-6), etc. TNF- α, PAI-1 and IL-6 are all proinflammatory cytokines, although IL-6 can exert anti-inflammatory properties in addition[1]. It has also been observed that leptin, resistin and TNF-α also impair insulin sensitivity and trigger atherogenesis[1]. These adipokines usually increase with adiposity and can have a detrimental role on an individual's health. Adiponectin, however, unlike other adipokines produced by adipose tissue, possesses anti-atherogenic properties and is found to decrease with increased adiposity. [1,2]

Several studies provide evidence of improvements in adipokine profiles with weight loss[3-6]. However, it remains unclear, whether changes in circulating

adipokines investigated to date contribute significantly to the beneficial effects in health associated with weight loss[7]. The extent of weight loss required to elicit such changes in adipokine levels remains unclear. In addition, the method by which the weight loss is achieved may also influence the adipokine response. Varady and co-workers [8], suggest that a minimum weight loss of 5% is required to have an effect on levels of key adipokines (adiponectin, leptin and resistin), however, this differs from other studies which report the need for a 10% weight loss for the modification in the levels of some adipokines (e.g. adiponectin) and that this varies with the degree of obesity[9].

Further studies are needed to clarify the relationship between changes in adipokine levels and the health benefits of weight loss, and whether specific dietary manipulations have differential effects on such changes. We hypothesised that the extent of weight loss rather than the macronutrient intake will determine the degree of change in adipokine levels.

We aimed to investigate the effect of weight loss on adipokine levels in individuals undergoing a low carbohydrate/high protein (LCHP) compared to a very low calorie diet (LighterLife UK Ltd (LL)) diet.

Methods

The present analysis is an ancillary study to that previously presented by Rolland et al [10] where the methods were previously described. Here, the drop outs were not included in this analysis as the goal was to evaluate the effect

of diet on adipokine levels and not an intention-to-treat clinical trial. In brief, patients referred to a Specialist Obesity Clinic were entered into a randomised controlled clinical trial (RCT) of differing dietary intervention in the management of obesity. Men and women older than 18 years of age and a BMI ≥ 35 kg/m^2 were included. Patients with a history of hepatic or renal disease, cancer, with current pregnancy/lactating, on anti-depressants or anti-obesity medication, or eating disorders were excluded. All patients who fulfilled these criteria were invited to take part in the study. This study included a three month screening period where patients were assigned to 600 calorie deficient diet (CDD) aiming to achieve a 5% percent weight loss. This was done to select patients who would not respond to a low fat, reduced energy approach and randomly assign them to a low carbohydrate/high protein (LCHP) or a very-low-calorie diet (VLCD)(LighterLife – LL) in a form of continuous method approach. Those who lost > 5% of their body weight were maintained on this approach for an additional 3 months. If weight loss was > 10% at this time, the CDD was continued for an additional 6 months. Completers data for this group were limited (n = 4) and, therefore, not included in this paper.

Those patients who failed to achieve the weight loss targets were randomly allocated to either LCHP or LL, and continued on the assigned diet for an additional 9 months (Figure 1). Patients on CDD who achieved a 5% weight loss at screening but did not achieve the 10% weight loss 3 months post-screening, were randomised to LL or LCHP but their data were omitted from

this analysis. All patients provided written informed consent. The study was approved by the North of Scotland Research Ethics Service.

<< INSERT FIGURE 1 HERE>>

Dietary intervention

Screening Period

Using the Schofield and the Harris Benedict Equations, 600-kcalories were removed from the patients' estimated daily energy intake. The amount of energy allowed was broken down into portions of different food groups [10]. Portion sizes were explained to the patients and written information was also provided. Patients were reviewed at 2, 4, 8 and 12 weeks when they were weighed, their weight loss progress was discussed and, were provided with dietary advice.

Randomisation and diet allocation

Patients randomised to LCHP were restricted to ≤40 g of carbohydrate per day. The energy intake ranged from 800-1500kcal where an 800 kcal diet was composed of 20% carbohydrate, 40% protein and 40% fat. Patients were given a booklet with information about which foods to eat and which to avoid. Examples of recipes were also provided. The diet was supplemented with multivitamins and minerals (Forceval, Alliance Pharmaceuticals, Chippenham, UK).

LighterLife is a VLCD which is administered in the shape of soups, shakes and bars to replace conventional food and provide a daily average of 550 kcal (36% carbohydrate, 36% protein and 28% fat and at least 100% RDAs for all micronutrients). Patients on it were advised to remain adequately hydrated while on the diet. LL has two distinctive stages: weight loss and food reintroduction. During each stage, patients attend weekly single-sex group meetings of 7-12 people for behaviour change therapy based on cognitive behaviour therapy and transactional analysis methodology delivered by a trained LL advisor. Patients were required to remain on the weight loss phase for a minimum of 3 months after which they were given a choice to continue for up to another 6 months or assigned to the food reintroduction phase. On average, patients who completed the study remained on the diet for 6.9 months (range: 4 - 9 months). For the food reintroduction phase, solid foods were reintroduced over a 12 week period where patients were slowly weaned off food packs while still receiving advice and support.

All patients came monthly to the trial centre to be weighed for the first 3 months and then every alternate month post-screening, resulting in 6 visits in 9 months. Additional support was provided via telephone and email.

Data Collection

Fat mass and fat free mass was estimated using bioelectrical impedance (Tanita BC-418 MA) (Tanita Corp., Arlington Heights, Illinois). Body

composition, and waist circumference were measured pre-screening, at screening, 3 and 9 months post-screening.

Blood samples were obtained after an overnight fast pre-screening, at screening, 3 and 9 months post-screening to measure fasting plasma glucose, insulin, adipokines including leptin, resistin, adiponectin, PAI-1 (active), IL-6, and TNF-α. Serum samples for adipokines and insulin were measured in duplicate by using commercially available immunoassay kits from Millipore using the Lincoplex system (St.Charles, MO). The protocol for measurement of insulin and all the adipokines was carried out as described by the manufacturer. The sensitivities are described in Table 1. Intra-assay variations ranged from 1.4-7.9 % coefficient of variation. High density lipoprotein cholesterol (HDL), triacylglycerol and fasting glucose were analysed in the Department of Clinical Biochemistry at NHS Grampian. Insulin resistance using fasting glucose and insulin values was calculated using the Homeostasis Model of Assessment of Insulin Resistance (HOMA-IR) where HOMA-IR = [insulin][glucose]/22.5.

<<INSERT TABLE 1 HERE>>

Statistical methods

All variables were assessed for normality using the Kolmogorov-Smirnov test. Any skewed data were log-transformed and subsequently assessed using parametric tests. For within group analysis, a paired t-test was used. For between-group analysis of changes, independent t-tests were used. Pearson

correlation was used to assess the relationship between changes in adipokines and changes in other continuous measurements. A Chi squared test was used to assess differences for dichotomous variables. Statistical tests were carried out using SPSS 15.0 for Windows software program (SPSS, Chicago, IL). As this is an ancillary analysis to a previously published study[10], the power for this analysis was carried out based on weight loss. A p-value of < 0.05 (two-tailed) was considered statistically significant. Based on the mean change (31.0 kg) and standard deviation (16.4 kg) for weight loss observed at 9 months [10], 14 patients in each group resulted in a >99% power for weight change.

Results

Data are presented as means ± standard deviation, and changes are expressed as the mean difference change from baseline ± standard deviations.

Baseline

Baseline characteristics are listed in Table 2. A total of 31 (14 in LL: 17 in LCHP) patients completed the study. There were significantly more men in the LL than the LCHP group (n = 5 and n = 1 respectively). Participants on LL also had significantly greater weight, BMI, waist circumference and fat free mass than the LCHP group.

<<INSERT TABLE 2 HERE>>

Weight, BMI, FFM were also significantly greater in the LL than the LCHP group (Table 2). When investigating the whole group, significant associations were observed between adiponectin, HDL and PAI-1. PAI-1 also had significant associations with insulin and HOMA-IR. Leptin was significantly associated with percentage body fat and TNF- α, while IL-6 was inversely associated with HDL (Table 3). There were no significant gender differences for the adipokines with the exception of leptin, where levels were significantly greater in women than men (46.7 ± 15.5 vs 28.2 ± 14.6, p = 0.014). This, however, was no longer significant after adjusting for percentage body fat (p = 0.089).

<<INSERT TABLE 3 HERE>>

Three month data

At 3 months, percentage weight loss for LCHP was -2.9% ± 4.2 and for LL was -18.4% ± 5.4 (p<0.0001). Weight, waist circumference, percentage body fat, fat mass, leptin, PAI-1, fasting glucose, HOMA-IR, triacylglycerols had all improved significantly in the LL group (Table 4). However, HDL and fat free mass decreased significantly from baseline in the LL group (Table 4).

A significant improvement from baseline to 3 months was observed for weight, percentage body fat, fat mass, insulin and HOMA-IR for the LCHP group (Table 4).

<<INSERT TABLE 4 HERE>>

Nine month data

At 9 months, percentage weight change for LCHP was -1.4% (1.0) and for LL was -23.8% ± 4.0 ($p<0.0001$). In the LL group, weight loss, waist circumference, percentage body fat, fat mass, fat free mass, leptin, PAI-1, fasting glucose, triacylglycerols were still significantly improved compared to baseline (Table 4). In addition, significant improvements in circulating levels of adiponectin, and HDL were observed but HOMA-IR was no longer significant (Table 4). Changes in adiponectin were inversely associated with changes in weight, BMI, fat mass, percentage body fat, waist circumference, IL-6 and TNF- α . Changes in leptin were associated with changes in weight, BMI, fat mass, waist circumference, fat free mass, and percentage body fat. Changes in leptin were inversely associated with HDL. Changes in TNF-α were associated with changes in IL-6, and inversely associated with changes in adiponectin. Changes in PAI-1 were associated with changes in fat mass (Table 5).

<<INSERT TABLE 5 HERE>>

There were no significant changes from baseline at 9 months for the LCHP group (Table 4). Changes in leptin, however, were inversely associated with changes in HDL ($r = – 0.573$, $p = 0.032$).

Overall, changes at 9 months for weight, waist circumference, fat mass, adiponectin, leptin, fasting glucose and HDL were significantly greater for LL than LCHP (Table 4).

Discussion

In the present study, there was a significant weight loss at 3 months for patients on both LL and LCHP. Significant weight loss at 9 months, however, was only maintained in the LL group. Changes in adiponectin and leptin were significantly greater for LL than LCHP which may be due to the greater weight loss and decrease in fat mass. Changes in TNF-α, IL-6, PAI-1 and resistin, however, did not differ significantly between the dietary groups at 9 months.

At baseline, we observed a trend for adiponectin to be inversely correlated with weight and BMI, but this did not reach significance, possibly due to the small sample size. Surprisingly, there was no significant inverse correlation between adiponectin and waist circumference which is unexpected, as other studies have demonstrated that waist circumference is a good correlate for adiponectemia [12,13]. Although the sample size was small, this may not be the reason for the lack of correlation of adiponectin to BMI and waist circumference. Mean BMI for the participants in the study was greater than 40 kg/m^2. There is evidence to suggest that the incidence of metabolic syndrome decreases beyond a BMI of 37.5 kg/m^2 [14].

Interestingly, our findings supported the evidence presented by Plaisance et al [15], where baseline adiponectin levels were strongly correlated with mean

HDL cholesterol. Association of moderately decreased risk of CVD with increased adiponectin is thought to be mediated in part by the effects of adiponectin on HDL, through parallel increases in both. However, how adiponectin affects HDL remains unknown [16]. In the LL group, changes in adiponectin were significant at 9 months but not at 3 months. Weight loss increased significantly from 3 to 9 months in this group which would suggest that a weight loss greater than 18.4% is required for statistically significant improvements in circulating adiponectin levels. Changes in adiponectin were inversely associated with fat mass, waist circumference, IL-6 and TNF-α at 9 months suggesting an improvement in inflammatory status with weight loss and the associated increase in adiponectin.

In the present study, leptin was significantly associated with percentage body fat at baseline, which is consistent with the previous findings [17]. There was no significant correlation found between leptin and waist circumference in the present study at baseline. This suggests that there may be a greater release of leptin from peripheral subcutaneous adipose tissue compared to visceral adipose tissue. Studies have shown that there are variations in leptin gene expression in adipose tissue depending on the site where it is deposited, where expression is greater in subcutaneous compared to visceral adipose tissue [18,19]. Alternatively, the lack of association between leptin and waist circumference may have been due to issues in the measurement of waist circumference in the grade III obese patients, a difficulty which is well recognised. Establishing the correct location and reading the tape in situ can be problematic. In addition, different protocols yield different results [20].

Leptin was significantly decreased at 3 and 9 months for the LL group. The changes in leptin were significantly associated with fat mass, percentage body fat, waist circumference. This is consistent with a number of studies in which leptin is shown to decrease in response to weight loss [21-24]. There was also an association in leptin change with waist circumference change at 9 months reflecting an overall loss of fat mass. In addition, despite the minimal weight loss for the LCHP group, changes in leptin were inversely associated with HDL at 9 months. This may suggest that even a small reduction in weight results in a beneficial trend for improvements in leptin levels which are likely to improve cardiovascular risk.

Levels of PAI-1 at baseline were associated with waist circumference, HOMA-IR and insulin, suggesting that PAI-1 is involved in insulin resistance as previously reported [25]. Weight loss has been found to reduce the levels of PAI-1 [26-28], indicating the influence of adipose tissue on the levels of this protein. Our study data are in support of this evidence where changes in PAI-1 were associated with changes in fat mass for the LL group at 9 months.

There was no evidence from the present study to support a strong link between resistin levels and weight. Of all the adipokines examined in this study, resistin appears to be the most controversial as the evidence appears to be equivocal and inconclusive [29-31]. Further human studies are required to confirm whether there is a relationship between resistin and obesity, including insulin resistance and type 2 diabetes mellitus.

Similarly, despite the fact that IL-6 has been found to be increased in obesity [32,33] and reduced in response to weight loss [34], no significant differences at baseline or in response to diet were observed here. Subcutaneous adipose tissue is thought to release ~ 30% of systemic IL-6, and visceral adipose tissue is thought to release even more. The lack of change, however, may be explained by the fact that only about 10% of total IL-6 produced by fat cells [35].

In addition, mean levels of TNF-α, did not significantly correlate with neither weight, waist circumference nor percentage body fat at baseline. These results were unexpected, as it is widely reported that TNF-α is linked to obesity [36]. Also, TNF-α did not show any significant decreases in response to weight loss or diet. However, in the study by Arvidsson et al [37], circulating levels of TNF-α did not show a significant difference after a mean weight loss of 7.5 % at 10 weeks. These authors concluded that adipose tissue only has a minor effect on the regulation of circulating TNF-α levels. Thus, TNF-α seems to be produced and act locally in human fat tissue [38], and there is no *in situ* release from adipose tissue into the blood [35]. A number of studies have observed no changes in IL-6, or TNF-α after significant reductions in weight (5–9 kg) in dietary and exercise interventions [39,40].

It was, however, interesting to observe that changes in IL-6 and TNF- α were significantly correlated at 9 months in the LL group. This could be explained by the relation between IL-6 and TNF- α where IL-6 exerts pro-inflammatory activity itself and by increasing TNF- α [1]. This would suggest that despite

weight loss not resulting in significant improvements in circulating levels of IL-6 and TNF- α, there appears to be an underlying clinically significant response where the reduction in weight results in a decrease in inflammation.

There is evidence that weight loss achieved using a VLCD results in similar changes in adipokine levels observed in response to bariatric surgery. Mitterberger et al [41] compared a group of patients for whom weight loss was achieved by dietary caloric restriction only to a group where caloric restriction was induced by bariatric surgery (3 gastric bypasses and 8 gastric bands). They reported that despite the fact that only 26 ± 7% weight loss was achieved in the dietary caloric restriction as compared to 43 ± 10% in the bariatric surgery group, changes in adipokines were not significantly different between the two groups. This may suggest an important role for the use of a weight loss approach such as the VLCD “instead of” bariatric surgery which is highly invasive, expensive and can lead to long-term vitamin and mineral deficiencies [42].

Although it remains unclear as to whether adipokines investigated to date are responsible for the beneficial effects in health associated with weight loss, it is clear from this study and several others, that there are important associations between weight loss, certain adipokines such as adiponectin, and leptin levels and cardiovascular disease risk. In addition, the extent of weight loss required to elicit the benefits as well as the effects of the method by which weight loss is obtained remains unknown. It can, however, be argued that there does seem to be a minimal weight loss required which was not achieved here in the LCHP and a maximal weight loss beyond which further improvements in

circulating adipokine levels are no longer observed as demonstrated in the study by Mitterberg et al [41]. Further research would include determining if there is a ceiling effect for adipokine change in response to weight loss. This would involve directly comparing adipokine changes in response to weight loss achieved by using a VLCD as compared to weight loss achieved using surgical approaches.

There were several limitations associated with the present study. There were significantly more men in LL than LCHP, however, this may not have been too limiting as there were no significant gender effects on adipokine when all patients were combined at baseline except for leptin, but it may have resulted in the significant differences between the two groups at baseline for weight, waist circumference and fat free mass. In addition, although the sample size provided a >99 % power for weight loss, the sample sizes may have been too small to observe associations and changes in adipokines, which would have been expected based on the literature.

Moreover, the use of multiplex assay has been criticised in the scientific literature [43]. The multiplexed bead immunoassay (allows the simultaneously detection of adipokines in small blood samples which may be particularly useful when samples are difficult to obtain). The use of this approach has been validated and found to be useful for leptin, adiponectin and insulin in the evaluation of changes in obesity markers following weight reduction[43]. The relationship between multiplexed bead immunoassay when compared to radio

immunoassay or enzyme-linked immunoassay (ELISA) for other adipokines such as resistin, IL-6 and TNF-α, however, were reported to be quite weak. These differences could be explained by differences in anti-body pairs and sample diluents as well as being affected by the low concentrations of these adipokines. In addition, assay sensitivity remains an issue when compared to ultra-sensitive ELISA methods [43]. However, the ability of multi-plexed assays to detect adipokines of a broader dynamic range than ELISAs as well as its greater cost-effectiveness, time efficient, requirement of smaller sample volume, and the removal of inter-assay variability suggest that this approach is still to be considered a powerful tool, albeit with the above limitations.

Conclusion

It would appear that the significant weight loss of 23.8% observed on LL resulted in significant improvements in circulating levels of leptin, PAI-1 and adiponectin. Changes in these adipokines possibly resulted in improvements in fasting glucose, triacylglycerols and HDL. This is most likely due to the overall weight loss achieved rather than macronutrient intake. Further research examining the adipokine response to weight loss using a very low calorie diet in comparison to surgical interventions would be beneficial to determine if the adipokine response to weight loss has a ceiling effect.

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Conflict of interest

Prof Iain Broom is the Medical Director for LighterLife.

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Table 1: Assay sensitivities for the measured adipokines.

Adipokine	**Sensitivity (pg/ml)**
Adiponectin	145.4
Insulin	50.9
Interleukin-6	1.6
Leptin	85.4
Plasminogen activator inhibitor-1 (active)	1.3
Resistin	4.5
Tumour necrosis factor -α	0.14

Table 2: Baseline characteristics of the two dietary interventions.

Variable	LL (n = 14)	LCHP (n = 17)	P
Age (years)	41.9 ± 6.5	45.8 ± 13.8	0.314
Gender			
male	5 (36)	1 (6)	0.036
female	9 (64)	16 (94)	
# of smokers	0 (0)	1 (6)	0.356
Weight (Kg)	129.5 ± 23.9	110.1 ± 10.6	0.012
BMI (Kg/m^2)	46.7 ± 9.0	40.6 ± 3.7	0.031
Waist circumference (cm)	127.5 ± 17.8	123.8 ± 10.6	0.031
Fat mass (Kg)	61.7 ± 18.8	52.6 ± 7.6	0.110
Fat free mass (Kg)	67.8 ± 14.3	57.4 ± 8.1	0.025
Percentage body fat (%)	47.2 ± 8.4	47.8 ± 4.9	0.797
Adiponectin (ng/ml)	20.3 ± 12.5	24.7 ± 14.4	0.384
IL-6 (pg/ml)	4.4 ± 2.9	2.9 ± 2.4	0.119
Leptin (ng/ml)	42.3 ± 20.3	43.5 ± 13.8	0.860
PAI-1 (active) (ng/ml)	52.5 ± 29.7	59.4 ± 54.0	0.926
Resistin (ng/ml)	21.1 ± 7.2	21.4 ± 18.2	0.164
TNFα (pg/ml)	4.3 ± 2.7	5.1 ± 2.0	0.549
Insulin (µUI/ml)	12.3 ± 8.2	14.6 ± 6.6	0.212
Fasting glucose (mmol/L)	5.3 ± 0.8	5.3 ± 0.6	0.912
HOMA-IR	3.1 ± 2.6	3.5 ± 1.8	0.619
HDL (mmol/L)	1.3 ± 0.2	1.4 ± 0.3	0.190
Triacylglycerol (mmol/L)	1.2 ± 0.7	1.6 ± 1.2	0.178

Values are means ± standard deviation; (percentages).

* Baseline differences were significantly different ($p<0.05$) between the two groups.

BMI, body mass index; HDL – high density lipoprotein cholesterol ; HOMA-IR – Homeostatic Model Assessmen-Insulin resistance; IL-6, interleukin-6; LCHP, low carbohydrate/high protein; LL, Lighterlife; PAI-1, plasminogen activator inhibitor – 1; TNFα – tumor necrosis factor alpha.

Table 3: Baseline correlations for all study participants.

	Adipo	IL-6	Leptin	PAI-1	Resistin	TNF- α	Insulin
Weight	-0.044	0.220	0.019	0.059	0.241	-0.079	0.090
BMI	-0.107	0.353	0.333	0.127	0.315	0.167	0.045
WC	0.033	0.143	-0.036	0.144	0.211	-0.124	0.379*
FM	-0.033	0.320	0.309	0.150	0.326	0.124	0.061
FFM	-0.069	-0.023	-0.333	-0.079	0.014	-0.277	0.074
% BF	0.084	0.256	0.478**	0.149	0.285	0.284	-0.014
Adipo	-	-0.128	0.086	-0.316	0.103	0.128	-0.289
IL-6	-0.128	-	0.220	0.109	0.056	0.252	0.231
Leptin	0.086	0.220	-	0.061	0.000	0.391*	0.257
PAI-1	-0.316	0.109	0.061	-	-0.022	-0.101	0.386*
Resistin	0.103	0.056	0.000	-0.022	-	-0.169	-0.099
TNF-α	0.128	0.252	0.391*	-0.101	-0.169	-	0.165
Insulin	-0.298	0.231	0.257	0.386*	-0.099	0.165	-
FG	-0.294	0.055	-0.119	0.223	0.123	0.287	0.331
HOMA	-0.344	0.141	0.207	0.399*	-0.049	0.212	0.979**
HDL	0.455*	-0.374*	0.053	-0.263	-0.062	0.154	-0.357
TAG	0.010	-0.188	-0.272	0.249	0.058	0.052	0.023

%BF, percent body fat; Adipo, adiponectin; BMI, body mass index; FG, fasting glucose; FM, fat mass; FFM, fat free mass; HDL – high density lipoprotein cholesterol ; HOMA – Homeostatic Model Assessment; IL-6, interleukin -6; PAI-1, plasminogen activator inhibitor – 1;TAG, triacylglycerol; TNFα – tumor necrosis factor alpha; WC – waist circumference.

*P <0.05
** P <0.01

Table 4: Changes in variables between baseline and 3 and 9 months (completers only)

Variable	Baseline	3 months	9 months	Change at 9 months	P (change between both studies at 9 months)
Weight (Kg)					
LL (n = 14)	129.5 ± 23.9	105.5 ± 20.1*	97.2 ± 20.8*	-32.3 ± 22.7	<0.0001
LCHP (n=17)	110.1 ± 10.6	107.1 ± 13.7*	108.7 ± 13.2	-1.3 ± 4.7	
BMI (Kg/m^2)					
LL	46.7 ± 9.0	38.1 ± 7.6	34.4 ± 9.2*	-12.3 ± 10.0*	0.001
LCHP	40.6 ± 3.6	39.5 ± 4.3	40.1 ± 4.1	-0.5 ± 1.8	
Waist circumference (cm)					
LL	127.5 ± 17.8	112.3 ± 17.5*	102.5 ± 10.4*†	-25.0 ± 17.7	0.001
LCHP	123.8 ± 10.6	121 ± 9.9	120.1 ± 10.1	-3.7 ± 8.0	
Fat mass (Kg)					
LL	61.7 ± 18.8	43.2 ± 14.7*	34.4 ± 18.3*†	-27.3 ± 19.0	<0.0001
LCHP	52.6 ± 7.6	50.3 ± 9.2*	52.1 ± 10.8	-0.5 ± 4.9	

Fat free mass (Kg)					
LL	67.8 ± 14.3	62.4 ± 12.7*	62.7 ± 13.5*	-5.1 ± 5.3	0.638
LCHP	57.4 ± 8.1	56.8 ± 9.2	56.6 ± 8.4	- 0.8 ± 2.8	
Percentage body fat (%)					
LL	47.2 ± 8.4	40.5 ± 8.7*	34.3 ± 12.9*†	-12.9 ± 9.4	<0.0001
LCHP	47.8 ± 4.9	46.7 ± 5.3*	47.7 ± 6.0	-0.1 ± 2.6	
Adiponectin (ng/ml)					
LL	20.3 ± 12.5	22.4 ± 7.8	36.1 ± 11.1*†	15.8 ± 17.1	0.003
LCHP	24.7 ± 14.4	23.9 ± 13.2	23.9 ± 13.2	-0.8 ± 6.2	
IL-6 (pg/ml)					
LL	4.4 ± 2.9	4.5 ± 5.3	4.4 ± 5.4	0.1 ± 4.4	0.716
LCHP	2.9 ± 2.4	3.1 ± 3.8	2.5 ± 1.7	-0.4 ± 2.5	
Leptin (ng/ml)					
LL	42.3 ± 20.3	26.1 ± 21.8*	24.7 ± 21.1*	-17.6 ± 24.3	0.049
LCHP	43.5 ± 13.8	41.1 ± 17.1	40.5 ± 14.7	-3.0 ± 9.2	

PAI-1 (active) (ng/ml)					
LL	52.5 ± 29.7	20.2 ± 11.1*	20.5 ± 19.3*	-32.0 ± 34.4	0.727
LCHP	59.4 ± 54.0	50.6 ± 43.3	49.0 ± 44.9	-10.5 ± 56.2	
Resistin (ng/ml)					
LL	21.1 ± 7.2	17.4 ± 7.0	16.7 ± 7.6	-4.4 ± 9.5	0.720
LCHP	21.4 ± 18.2	16.4 ± 11.0	16.0 ± 7.5	-5.5 ± 15.8	
TNFα (pg/ml)					
LL	4.3 ± 2.7	4.1 ± 2.6	4.5 ± 2.9	0.3 ± 2.8	0.928
LCHP	5.1 ± 2.0	5.1 ± 2.7	5.2 ± 2.0	0.2 ± 1.5	
Insulin (µUI/ml)					
LL	12.3 ± 8.2	10.6 ± 12.7	10.9 ± 7.4	-3.7 ± 7.4	0.902
LCHP	14.6 ± 6.6	12.1 ± 6.5*	12.2 ± 7.3	- 4.0 ± 11.1	
Fasting glucose (mmol/L)					
LL	5.3 ± 0.8	4.6 ± 0.5*	4.7 ± 0.3*	-0.7 ± 0.9	0.034
LCHP	5.3 ± 0.6	5.4 ± 0.6	5.2 ± 0.5	-0.1 ± 0.5	
HOMA-IR					
LL	3.1 ± 2.6	2.1 ± 2.5*	2.2 ± 1.5	-1.6 ± 2.4	0.832

LCHP	3.5 ± 1.8	2.9 ± 1.6*	2.8 ± 1.8	-1.1 ± 2.7	
HDL (mmol/L)					
LL	1.3 ± 0.2	1.2 ± 0.1*	1.5 ± 0.2*†	0.2 ± 0.2	0.018
LCHP	1.4 ± 0.3	1.4 ± 0.3	1.4 ± 0.4	0.0 ± 0.2	
Triacylglycerol (mmol/L)					
LL	1.2 ± 0.7	0.9 ± 0.2*	0.7 ± 0.2*	-0.4 ± 0.8	0.070
LCHP	1.6 ± 1.2	1.5 ± 0.9	1.4 ± 0.9	-0.2 ± 0.7	

Values are means ± standard deviation.

* Indicates a significant difference ($p<0.05$) from baseline.

† Indicates a significant difference ($p<0.05$) from 3 months.

BMI, body mass index; IL-6, interleukin -6; PAI-1, plasminogen activator inhibitor – 1; TNFα – tumor necrosis factor alpha; HOMA-IR – Homeostatic model assessment-insulin resistance; HDL – high density lipoprotein; LDL – low density lipoprotein; TC – total cholesterol.

Table 5: Correlations for changes from baseline to 9 months for the LighterLife group.

	Adipo	IL-6	Leptin	PAI-1	Resistin	TNF- α	Insulin
Weight	-0.560*	0.213	0.743**	0.505	-0.072	0.158	0.224
BMI	-0.612**	0.258	0.730*	0.462	-0.147	0.265	0.126
WC	-0.578*	0.217	0.720**	0.488	-0.064	0.158	0.105
FM	-0.637*	0.282	0.695**	0.558*	-0.064	0.237	0.204
FFM	-0.119	-0.117	0.694**	0.165	-0.081	-0.189	0.296
% BF	-0.681**	0.317	0.635*	0.531	-0.007	0.241	0.209
Adipo	-	-0.868**	-0.392	-0.407	-0.076	-0.740**	0.317
IL-6	-0.868**	-	0.209	0.186	0.220	0.779**	-0.460
Leptin	-0.392	0.209	-	0.474	-0.133	0.003	0.596
PAI-1	-0.407	0.186	0.474	-	-0.330	-0.193	0.544
Resistin	-0.076	0.220	-0.133	-0.330	-	0.141	-0.049
TNF-α	-0.740**	0.779**	0.003	-0.193	0.141	-	-0.466
Insulin	0.317	-0.460	0.596	0.544	-0.049	-0.466	-
FG	-0.427	0.177	0.439	0.238	-0.114	0.294	-0.115
HOMA	0.144	-0.337	0.708	0.652	-0.73	-0.349	0.917**
HDL	-0.506	-0.171	-0.573*	-0.508	-0.332	0.046	-0.412
TAG	0.531	0.306	0.336	0.437	0.105	0.130	0.417

%BF, percent body fat; Adipo, adiponectin; BMI, body mass index; FG, fasting glucose; FM, fat mass; FFM, fat free mass; HDL – high density lipoprotein cholesterol ; HOMA – Homeostatic Model Assessment; IL-6, interleukin -6; PAI-1, plasminogen activator inhibitor – 1;TAG, triacylglycerol; TNFα – tumor necrosis factor alpha; WC – waist circumference.

*P <0.05
** P <0.01