Running title: Preliminary findings from the EVASYON study

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Changes in cardiometabolic risk factors, appetite-controlling hormones and cytokines after a treatment programme in overweight adolescents: preliminary findings from the EVASYON study

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ABSTRACT
**Objective:** We investigated the effects of the EVASYON programme on body fatness, cardiometabolic risk factors, gut appetite-controlling hormones and serum levels of cytokines in adolescents with overweight or obesity (OW/OB). **Methods:** The study comprised 13 boys (10 obese) and 12 girls (8 obese), aged 13 to 16 years, from a Madrid Hospital. The EVASYON programme was based on a calorie-restricted diet (10-40%), increased physical activity (at least 60 min/day 5 days a week), psychological therapy and nutritional education for 13-month. Anthropometric and blood pressure measurements were measured before and after intervention. Serum glucose, total cholesterol, high-density lipoprotein cholesterol, triglycerides, leptin, total peptide YY and insulin levels were determined before and after intervention. Serum levels of cytokines IL-1β, IL-2, IL-4, IL-5, IL-6, IL-8, IL-10 and TNF-α were also assessed before and after intervention. **Results:** A decrease in body mass index (BMI), BMI z-score, skinfolds (triceps, biceps, subscapular, thigh, and calf), sum of 6 skinfolds and body circumferences (arm relaxed and flexed, waist, hip and proximal thigh) values were observed after the intervention programme (all *P*<0.05). In addition, diastolic blood pressure also decreased (*P*<0.05). A decrease in serum leptin levels (–48.4%, *P*<0.001) was observed after intervention without changes in total peptide PYY and insulin levels. Levels of IL-8, IL-10 and TNF-α also decreased (all *P*<0.05) after the intervention programme. **Conclusions:** These preliminary results evidence that the EVASYON programme may improve body fat, leptin, and some pro-inflammatory cytokines in adolescents with OW/OB.

**Key words:** adolescents; obesity; metabolic syndrome; gut hormones; cytokines.

**INTRODUCTION**
Obesity is the most prevalent metabolic disease in adult humans, which has been associated with cardiometabolic risk factors, such as low-grade inflammation, insulin resistance, hypertension and dislipidemia (1,2). Since some of these cardiometabolic risks have been already identified in obese children and adolescents, early obesity underlies the metabolic groundwork for adult cardiovascular diseases (3-6).

In this context, adipose tissue is a rich source of many immune-related mediators (e.g. cytokines such as tumour-necrosis factor-alpha [TNF-α] and interleukin-6 [IL-6]) that are involved in the inflammatory response (4-6), mediating cardiometabolic disorders (7). Furthermore, adipose tissue is currently regarded as an active endocrine organ that, in addition to regulating fat mass and energy homeostasis, it releases a large number of bioactive mediators modulating appetite and metabolism (8). Some of these bioactive mediators are hormones called adipokines and include leptin, adiponectin, peptide YY (PYY), among others (9,10). Recent data also suggest that these hormones have immunomodulating effects involving inflammatory processes (8-10).

In this sense, the EVASYON (Development, implementation and evaluation of the efficacy of a therapeutic programme for adolescents with overweight and obesity: integral education on nutrition and physical activity) programme comprised a long-term intervention with calorie-restricted diet, physical activity, psychological therapy and nutritional education in adolescents with overweight or obesity (OW/OB) in hospital settings from Spain. Since inflammation associated with obesity declines after weight loss in adults (4), we hypothesized that a programme aimed to lose fat mass might modify specific obesity-associated markers of inflammation in adolescents. Therefore, this preliminary study was performed to characterize the effects of the EVASYON treatment programme on body fat, cardiometabolic risk factors, gut appetite-controlling hormones and cytokines levels in adolescents with OW/OB.

METHODS AND PROCEDURES
Study design and participants

The EVASYON study is a multidisciplinary treatment programme study conducted in 5 hospitals from 5 Spanish cities (Granada, Madrid, Pamplona, Santander and Zaragoza) in OW/OB adolescents. The complete and detailed methodology of the EVASYON Study has been described elsewhere (11). Briefly, the EVASYON treatment programme was conducted in small groups (8-10 participants) during 13-month including twenty visits within two specific stages: 1) Intensive intervention (9 visits): participants visited the hospitals weekly for 2 months and one-week objectives were defined; 2) Extensive intervention (11 visits): participants visited the hospital monthly during 11 months. In this stage, objectives for the adolescents were to be accomplished in one month's time.

The EVASYON intervention included calorie-restricted diet (10 to 40%), increased physical activity (at least 60 min/day 5 days a week), psychological therapy and nutritional education for these 13 months. Paediatricians explained the patients several motivational strategies, life and time management strategies including physical activity and sedentary behaviour recommendations, adequate sleep time, nutritional advice, family involvement, among others.

Adolescents included in the EVASYON treatment met the inclusion criteria for participating: i) To be aged 13 to 16 years; ii) To be OW/OB in agreement with the International Obesity Task Force age- and sex-specific body mass index (kg/m², BMI) values (12); iii) To be Spanish or to have been educated in Spain; iv) To be free of other diagnosed disease. Moreover, adolescents in pharmacological treatment or diagnosed with anorexia, bulimia or other eating disorder, except binge eating disorder, were excluded.

In order to evaluate the long-term effect of the programme, we analyzed preliminary results corresponding to basal point (before the intervention) and after intensive and extensive phases of interventions (2+11 months) in OW/OB adolescents from the Madrid Hospital.
A total of 31 adolescents (13 girls) finished the EVASYON programme in this hospital. This study encompassed 13 boys and 12 girls (n=25) with valid data on anthropometry and blood samples before intervention and the end of the EVASYON treatment. These adolescents maintained an apparently good health status and did not consume any medications during the study.

The study was conducted in accordance with the ethical rules of the Helsinki Declaration (Hong Kong revision, September 1989, in Edinburgh in 2000 and in Korea in 2008), following the EEC Good Clinical Practice guidelines (document 111/3976/88 of July 1990) and current Spanish law, which regulates clinical research in humans (Royal Decree 561/1993 regarding clinical trials). Informed consent was obtained from all adolescents and their parents, and the study was approved by the local ethics committees.

**Anthropometry measurements**

Body weight was measured without shoes and with light clothing to the nearest 0.05 kg by using a standard beam balance. Body height was measured using a stadiometer SECA 714 (range, 60-200 cm). BMI z-scores were calculated as a function of the subject’s obesity degree when compared with BMI local reference standards (13). Skinfold thicknesses were measured on the left side of the body (14) to the nearest 0.1 mm with a skinfold caliper (Caliper Holtain; Holtain Ltd., Walles, UK) at triceps, biceps, subscapular, suprailiac, thigh, and calf. The five circumferences (arm relaxed, arm flexed, waist, hip and proximal waist) were measured in centimetres with an inelastic tape to the nearest millimetre. All the anthropometric variables were measured in order, three times and averaged. For all the anthropometric measurements, intraobserver reliability was >95% and interobserver reliability was >90%. In the present study, the sum of 6 skinfolds and waist circumference were used as indicators of total and central body fat, respectively.
Resting blood pressure

Blood pressure was measured using a validated digital automatic blood pressure monitor (Omron M6, Omron Health Care Co., Ltd., Kyoto, Japan) according to the International Protocol of the European Society of Hypertension (15).

Blood sampling

Blood samples were taken twice for each subject before and after the intervention (13-month). After an overnight fast (12-hour fast), subjects went to the hospital for blood sampling at 8:00 a.m. Blood samples were collected by puncture of the cubital vein. For biochemical analyses, the blood was collected in SST-Vacutainer (BD) and serum was separated by centrifugation at 3000 rpm for 15 min at 22-24ºC, divided into aliquots, and frozen and stored at –80ºC until withdrawn for analysis.

Biochemical parameters. Total cholesterol, HDL-cholesterol, triglycerides, and glucose were analysed by a biochemical autoanalyser (Olympus model AU2700). All serum analysis were performed at the end of the study, in order to have the three samples from each subject analysed in the same run, to avoid systematic errors. Coefficients of variance of lipid variables were 2% for total cholesterol, 2% for HDL-cholesterol and 3% for triacylglycerols. VLDL-cholesterol and LDL-cholesterol were calculated from existing cholesterol, HDL-cholesterol, and triglycerides values (16).

Appetite-controlling hormones. Serum levels of leptin, total peptide YY, insulin and adiponectin were measured by Luminex-100 IS (Integrated System: Luminex Corporation, Austin, TX, USA) using the human gut hormone multiplex immunoassay kit (HGT-68K) and Human CVD1 kit (HCVD1-67AK). The intra- and inter-assay precision coefficients of
variation were: 7% and 9% respectively for leptin; 5% and 10% respectively for total peptide YY; 4% and 6% respectively for insulin; and 9% and 16% respectively for adiponectin.

**Serum cytokines.** Determination of serum cytokines IL-1β, IL-2, IL-4, IL-5, IL-6, IL-8, IL-10 and TNF-α was performed on the Luminex-100 IS (Integrated System: Luminex Corporation, Austin, TX, USA) using the multiplex assay kit Linco High Sensitivity Human Cytokine Panel Lincoplex, 96 Well Plate Assay (HSCYTO-60SK), manufactured by Linco Research, Inc., MO, USA. Multianalyte profiling calibration microspheres for classification and reporter readings, as well as sheath fluid were also purchased from Luminex Corporation. Acquired fluorescence data were analyzed by the Luminex-100 IS v.2.3. All analyses were performed according to the manufacturer's protocols. The intra- and inter-assay precision coefficients of variation were: 3% and 2%, respectively for IL-1β; 4% and 8%, respectively for IL-2; 4% and 9%, respectively for IL-4; 5% and 14%, respectively for IL-5; 4% and 5%, respectively for IL-6; respectively for IL-8; 3% and 12%, respectively for IL-10; and 4%, respectively for TNF-α.

**Statistical analyses**

Data were assessed for normality and homogeneity of variance, and are expressed as mean ± standard deviation (SD). Student’s t test was used to compare means between before and after intervention (13-month). Due to non-normal distribution, the Wilcoxon test was applied to appetite-controlling hormones and cytokine values to compare means between before and after intervention. Effect sizes (ES) were also calculated using Cohen’s $d$ as a measure of ES that reflects the magnitude of the difference between groups in SD units. Cohen's $d$ is computed by subtracting the average score for the control group (before the intervention) from the average score for the treatment group (after the EVASYON treatment programme), and then dividing the difference by the SD on the outcome measure for the
sample (17). We used the standard criteria for ES of small \((d=0.20)\), medium \((d=0.50)\), and large effects \((d=0.80)\). We employed these criteria along with the results of the statistical tests in evaluating the impact of the previous results of EVASYON programme presented in the present work. Analysis was performed using SPSS software v.17. Statistical significance was set at \(P<0.05\).

RESULTS
Adolescents included in the final analysis were 14.1 ± 1.1 years old, and the prevalence of obesity at baseline was 72% (10 boys and 8 girls). These subjects showed marked differences in body fat characteristics after the intervention programme (Table 1). Thus, significant decreases in BMI mean values (–6.8%) and BMI z-score (–28.0%) were found after the intervention (all \( P < 0.05 \)). Levels of body fat measured by anthropometry at triceps, biceps, subscapular, thigh, and calf also decreased after the intervention (all \( P < 0.05 \)). The main indicators of total and central body fat, that is, the sum of 6 skinfolds (–17.8%) and waist circumference (–3.8%), also significantly decreased after the intervention (both \( P < 0.05 \)).

Regarding cardiometabolic risk factors included in the metabolic syndrome definition (Table 2), a more favourable profile was found in all of them, but only diastolic blood pressure (–7.6%) significantly decreased after the intervention programme (\( P = 0.018 \)). No significant changes on adiponectin, total peptide YY and insulin levels were observed after the intervention (Table 3). In contrast, a decrease (\( P < 0.001 \)) on serum leptin levels (–48.4%) was found after the intervention (Table 3). Levels of IL-8 (–34.4%), IL-10 (–39.8%) and TNF-\( \alpha \) (–21.5%) also significantly decreased (all \( P < 0.05 \)) after the intervention programme (Table 4).

DISCUSSION
These preliminary results of the EVASYON treatment programme indicate a clinically relevant body fat reduction after the intervention programme in adolescents with OW/OB. This outcome has been observed mainly in the sum of 6 skinfolds (large ES) as an indicator of total body fat. Adolescence is characterised by an intense growth and development and in this sense, it is important to stress the fact that although body weight did not decrease after intervention, the decrease in BMI values observed is attributable to the increase in the body height observed. These results also point out that in adolescents with OW/OB, the improvement of body composition induced by the EVASYON intervention programme was concomitant with decreased serum concentrations of leptin, IL-8, IL-10 and TNF-α.

High serum leptin levels have been positively correlated with the components of the metabolic syndrome in obesity during adolescence (18,19). The reduction of leptin levels observed in the present study after body composition improvement is in agreement with other trials (20,21). Although these studies did not evaluate the ES, the leptin decrease observed after the EVASYON treatment programme showed a medium to large ES. Elloumi and co-workers (20) evaluated the effect of 2-month weight-loss programme (based on energy restriction and training at the point of maximum lipid oxidation) on plasma levels of adiponectin and leptin, in 21 obese adolescent boys. The authors found that the adolescents who improved their body composition showed an increase in plasma adiponectin concentrations (+74%) and a decrease in plasma leptin levels (–39%). In addition, a decrease in plasma leptin levels (–70%) together with an increase in plasma adiponectin concentrations (+27%) have been found after a 9-month multidisciplinary weight-reduction programme with lifestyle education, moderate energy restriction and regular physical activity (21). Likewise, our results have not reflected the increase in adiponectin levels previously reported by these two studies and others (20-23), and seem to be in disagreement with the favourable body composition changes observed after the intervention programme.
On the other hand, high means of BMI and waist circumference values have been highly associated with elevated circulating levels of IL-6 and IL-8 (24). Since elevated levels of pro-inflammatory cytokines correlate with obesity in adolescents confirming the presence of early phases of atherosclerosis risk (25), the decrease in serum IL-8 and TNF-α concentrations (medium to large ES) observed in the current study after the intervention, might suggest an attenuation of inflammation related risk factors.

To our knowledge, only a small number of studies concerning changes on serum cytokine levels after intervention obesity programmes in adolescents are available. Thus, a significant decrease in circulating IL-6 levels (−48%), associated with weight loss and reduction of fat mass, have been reported (26) after short-term (3 weeks) under energy restriction and exercise programme, in 49 obese adolescents. On the contrary, our results have not reflected a significant decrease in IL-6 levels (−36%) after the intervention programme. This conflicting outcome might be due to, for example, the differences in (i) the duration of the interventions (3-week vs 13-month); (ii) IL-6 levels at baseline (3.9±4.7 vs 3.3±3.0 pg/mL); (iii) laboratory methods (ELISA vs Luminex); (iv) and sample sizes (49 vs 25 adolescents).

Although several authors have found no changes in TNF-α in obese adolescents or adults after weight loss (27-29), other studies in adults are in agreement with our results showing decreased TNF-α levels after weight loss (30-34). The release of pro-inflammatory biomarkers might be one mechanism through which obesity is linked to increased leptin values (7,10), and the current study concur with this hypothesis since leptin decreases were concurrent with IL-8 and TNF-α decreases after intervention.

Another conflicting finding in the present study is the decreased IL-10 levels observed after our intervention programme. On the contrary, increased serum IL-10 levels have been found after weight loss in obese adults (35). Since activated adipose tissue increases the
synthesis of pro-inflammatory cytokines while regulatory cytokines, such as IL-10 are decreased (36); the decrease in IL-10 values observed after intervention seems to be contradictory. In any case, no studies evaluating IL-10 cytokine after a weight loss programme are available in adolescents.

Our results have shown relevant results. Obese subjects go on to display a characteristic profile of hypertension, reduced HDL-cholesterol, and increased levels of LDL-cholesterol, triglycerides, and insulin resistance (metabolic syndrome), even during childhood (37-39). In another study, after two-months of a physical-endurance and diet-restriction programme in 24 obese adolescent boys, reductions in BMI and waist circumference (~13%) were associated with decreased plasma triglycerides, LDL-cholesterol and total cholesterol levels (40). Although a decrease in diastolic blood pressure was observed, the present study has not reflected changes on cardiometabolic risk factors such as systolic blood pressure and lipid profile after body composition reduction. The different findings found among studies might be partially explained by the specific characteristics of each study (e.g. study duration, sample size) as commented above. For example, other authors have detected an improvement on lipid profile after decreasing BMI in 37 obese children who participated in a 12-month intervention programme called "Obeldicks" (41). Although the duration of this programme was one year, in this case, the age differences of the subjects involved in each study (mean age 11 vs 14 years) could support the differences between their lipid profile results and our current findings.

The novelty of the current study is that it is the first long-term study (13-month) concerning the relationships between serum gut appetite-controlling hormones, serum cytokines and changes in body composition in OW/OB adolescents. One limitation of the present study includes the relative small sample size which could reduce the significance of the detected changes and the ES. In addition, body fat was obtained by anthropometric
measurements and other methods (e.g. dual energy X-ray absorptiometry, air displacement plethysmography, bioelectric impedance) may be more accurate for assessing body composition changes. In any case, these preliminary results point out to a prominent body fat reduction after the intervention programme, suggesting the potential usefulness of implementing the EVASYON intervention programme for weight management in adolescents with OW/OB. Future studies including the whole sample recruited in the EVASYON study must analyse the role of important determinants (e.g. sexual maturation, socioeconomic status, gene-environmental interactions) on the changes in body fat and cardiometabolic risks during the EVASYON treatment programme.

In conclusion, our preliminary results indicate that the EVASYON programme may produce moderate to large treatment effects for body fat reduction in adolescents with OW/OB. Although the body composition improvement did not improve all the classic metabolic syndrome components, these preliminary results suggest that the EVASYON programme may reduce earlier cardiometabolic risk factors such as leptin, IL-8 and TNF-α levels, contributing to prevent future cardiovascular events. Nevertheless, further research is required to examine whether this improvement in the inhibition of cardiometabolic risk factors (mainly by an earlier decline in low-grade systemic inflammation) will eventually lead to a clinical benefit associated with an improvement on body composition in children and adolescents.

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**DISCLOSURE**

The authors declare that they have no competing interests.

**REFERENCES**


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Adolescents in Madrid with overweight or obesity

Screening
N=42
(16 girls)

EVASYON Inclusion/Exclusion criteria

EVASYON treatment programme
n=35
(15 girls)

GROUP I
n=6
(3 girls)

GROUP II
n=9
(2 girls)

GROUP III
n=10
(6 girls)

GROUP IV
n=10
(4 girls)

GROUP I
n=6
(3 girls)

GROUP II
n=7
(2 girls)

GROUP III
n=9
(5 girls)

GROUP IV
n=9
(3 girls)

RECRUITMENT IN A HOSPITAL FROM MADRID HEALTH CARE SERVICE

INTENSIVE INTERVENTION
(1st-9th visit)
1 visit weekly
(2 months)

EXTENSIVE INTERVENTION
(10th-20th visit)
1 visit monthly
(11 months)
<table>
<thead>
<tr>
<th></th>
<th>Before the intervention</th>
<th>After the EVASYON treatment programme</th>
<th>Cohen's d</th>
</tr>
</thead>
<tbody>
<tr>
<td>Weight (kg)</td>
<td>87.0 ± 14.6</td>
<td>84.7 ± 16.5</td>
<td>0.15</td>
</tr>
<tr>
<td>Height (cm)</td>
<td>166.3 ± 7.1</td>
<td>170.4 ± 7.7***</td>
<td>0.56</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>31.4 ± 4.4</td>
<td>29.2 ± 5.0***</td>
<td>0.47</td>
</tr>
<tr>
<td>BMI z-score</td>
<td>4.7 ± 1.9</td>
<td>3.4 ± 1.9***</td>
<td>0.70</td>
</tr>
<tr>
<td>Biceps skinfold (mm)</td>
<td>18.6 ± 5.1</td>
<td>13.4 ± 5.5**</td>
<td>0.99</td>
</tr>
<tr>
<td>Triceps skinfold (mm)</td>
<td>26.1 ± 3.4</td>
<td>22.6 ± 7.0**</td>
<td>0.64</td>
</tr>
<tr>
<td>Subscapular skinfold (mm)</td>
<td>29.0 ± 8.6</td>
<td>25.3 ± 8.3*</td>
<td>0.44</td>
</tr>
<tr>
<td>Suprailiac skinfold (mm)</td>
<td>29.3 ± 3.5</td>
<td>27.0 ± 7.2</td>
<td>0.41</td>
</tr>
<tr>
<td>Thigh skinfold (mm)</td>
<td>37.2 ± 3.4</td>
<td>30.2 ± 8.1***</td>
<td>1.14</td>
</tr>
<tr>
<td>Calf skinfold (mm)</td>
<td>37.5 ± 4.7</td>
<td>28.2 ± 5.9***</td>
<td>1.74</td>
</tr>
<tr>
<td>Sum of 6 skinfolds (mm)</td>
<td>177.9 ± 18.8</td>
<td>147.0 ± 36.2***</td>
<td>1.08</td>
</tr>
<tr>
<td>Arm circumference (cm)</td>
<td>35.08 ± 3.6</td>
<td>33.4 ± 5.0**</td>
<td>0.40</td>
</tr>
<tr>
<td>Flexed arm circumference (cm)</td>
<td>35.8 ± 3.6</td>
<td>34.6 ± 4.9**</td>
<td>0.28</td>
</tr>
<tr>
<td>Waist circumference (cm)</td>
<td>101.2 ± 10.4</td>
<td>97.4 ± 13.5*</td>
<td>0.31</td>
</tr>
<tr>
<td>Hip circumference (cm)</td>
<td>112.6 ± 11.5</td>
<td>108.6 ± 11.0**</td>
<td>0.36</td>
</tr>
<tr>
<td>Proximal thigh circumference (cm)</td>
<td>70.4 ± 6.4</td>
<td>67.8 ± 7.7**</td>
<td>0.36</td>
</tr>
</tbody>
</table>

Values expressed as mean ± SD values. *P<0.05, **P<0.01, ***P<0.001, denotes statistical significance between baseline and after the intervention. Student’s t test.
Table 2. Cardiometabolic risk factors before (baseline) and after the intervention (n=25)

<table>
<thead>
<tr>
<th>Risk Factor</th>
<th>Before the intervention</th>
<th>After the EVASYON treatment programme</th>
<th>Cohen's d</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fasting glucose (mg/dL)</td>
<td>98.4 ± 8.1</td>
<td>95.9 ± 5.8</td>
<td>0.35</td>
</tr>
<tr>
<td>Total cholesterol (mg/dL)</td>
<td>146.2 ± 23.2</td>
<td>143.8 ± 24.6</td>
<td>0.10</td>
</tr>
<tr>
<td>Triglycerides (mg/dL)</td>
<td>56.7 ± 22.6</td>
<td>53.3 ± 31.1</td>
<td>0.13</td>
</tr>
<tr>
<td>HDL-Cholesterol (mg/dL)</td>
<td>45.1 ± 10.1</td>
<td>45.8 ± 10.4</td>
<td>0.07</td>
</tr>
<tr>
<td>LDL-Cholesterol (mg/dL)</td>
<td>89.8 ± 18.4</td>
<td>87.3 ± 17.5</td>
<td>0.14</td>
</tr>
<tr>
<td>Systolic blood pressure (mm Hg)</td>
<td>130.4 ± 17.6</td>
<td>127.4 ± 3.8</td>
<td>0.24</td>
</tr>
<tr>
<td>Diastolic blood pressure (mm Hg)</td>
<td>70.2 ± 8.8</td>
<td>65.4 ± 5.4*</td>
<td>0.67</td>
</tr>
</tbody>
</table>

Values expressed as mean ± SD values. *P<0.05, denotes statistical significance between baseline and after the intervention. Student’s t test.
<table>
<thead>
<tr>
<th></th>
<th>Before the intervention</th>
<th>After the EVASYON treatment programme</th>
<th>Cohen's $d$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Leptin (ng/mL)</td>
<td>22.0 ± 12.0</td>
<td>14.1 ± 10.1 *</td>
<td>0.71</td>
</tr>
<tr>
<td>Insulin (pg/mL)</td>
<td>488.6 ± 366.7</td>
<td>490.7 ± 444.2</td>
<td>0.01</td>
</tr>
<tr>
<td>Total peptide YY (pg/mL)</td>
<td>89.6 ± 47.0</td>
<td>87.6 ± 58.1</td>
<td>0.04</td>
</tr>
<tr>
<td>Adiponectin (ng/mL)</td>
<td>5.5 ± 8.7</td>
<td>5.4 ± 8.4</td>
<td>0.01</td>
</tr>
</tbody>
</table>

Values expressed as mean ± SD values. *$P$<0.05, denotes statistical significance between baseline and after the intervention. Wilcoxon test.
**Table 4.** Serum cytokines before (baseline) and after the intervention (n=25)

<table>
<thead>
<tr>
<th>Cytokine</th>
<th>Before the intervention</th>
<th>After the EVASYON treatment programme</th>
<th>Cohen's $d$</th>
</tr>
</thead>
<tbody>
<tr>
<td>IL-1β (pg/mL)</td>
<td>1.1 ± 2.8</td>
<td>0.6 ± 1.2</td>
<td>0.21</td>
</tr>
<tr>
<td>IL-2 (pg/mL)</td>
<td>3.0 ± 5.3</td>
<td>2.2 ± 4.6</td>
<td>0.17</td>
</tr>
<tr>
<td>IL-6 (pg/mL)</td>
<td>3.3 ± 3.0</td>
<td>2.1 ± 1.4</td>
<td>0.50</td>
</tr>
<tr>
<td>IL-4 (pg/mL)</td>
<td>5.2 ± 4.7</td>
<td>4.8 ± 3.9</td>
<td>0.10</td>
</tr>
<tr>
<td>IL-8 (pg/mL)</td>
<td>10.0 ± 6.2</td>
<td>6.5 ± 3.8 *</td>
<td>0.66</td>
</tr>
<tr>
<td>IL-10 (pg/mL)</td>
<td>17.6 ± 9.0</td>
<td>13.4 ± 6.7 *</td>
<td>0.52</td>
</tr>
<tr>
<td>TNF-α (pg/mL)</td>
<td>10.2 ± 4.4</td>
<td>8.0 ± 2.7 *</td>
<td>0.60</td>
</tr>
</tbody>
</table>

Values expressed as mean ± SD values. TNF-α: tumour necrosis factor-α. *$P<0.05$, denotes statistical significance between baseline and after the intervention. Wilcoxon test.