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Correlations between modest weight loss and leptin to adiponectin ratio, insulin and leptin resensitization in a small cohort of Norwegian individuals with obesity

Victoria T. Isaksen [a,*,1], Maria A. Larsen [a], Rasmus Goll [a,b], Eyvind J. Paulssen [a,b], Jon R. Florholmen [a,b]

[a] Research group of Gastroenterology and Nutrition, Department of Clinical Medicine, UiT The Arctic University of Norway, P. O. Box 6050, Langnes, N-9037 Tromsø, Norway
[b] Department of Gastroenterology, University Hospital of North Norway, Tromsø, Norway

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ABSTRACT

Background: Weight loss is important to reduce the risk of metabolic complications in obese individuals, in whom dysregulated adipokines play a central role. This study aims to investigate whether dysregulated adipokines and postprandial triglycerides (TG) improve with a modest weight loss.
Methods: Individuals with obesity (BMI ≥ 30 kg/m^2) were recruited among patients at the University Hospital of North Norway and the Stamina Health weight loss rehabilitation program. We measured resting energy expenditure (REE), and calculated the Homeostasis Model Assessment of Insulin Resistance (HOMA-IR), leptin to adiponectin (L:A) ratio, indirect leptin sensitivity (REE:leptin ratio), postprandial TG clearance at 6 h, and TG response before and after weight loss. The goal of the weight loss intervention was a loss of ≥5 % of initial total body weight.
Results: 28 participants completed the study, of which 13 lost ≥ 5 % body weight and 18 lost <5 % body weight. HOMA-IR (−23.1 %), REE:leptin ratio (+80.1 %) and L:A ratio (−45.7 %) significantly improved with weight loss, whereas there was no improvement of postprandial TG response or clearance. No significant changes were observed in the non-weight loss group.
Conclusion: The data are consistent with the general concept that modest weight loss in obese patients may restore metabolic regulation by improving L:A ratio and insulin and leptin sensitivity.

1. Background

Overweight and obesity are important risk factors for morbidity and mortality (O'Neill and O'Driscoll, 2015; WHO, 2020), mainly because of their association with metabolic dysfunction and increased risk of cardiovascular disease (CVD), cancer, and type 2 diabetes mellitus (T2DM) (O'Neill and O'Driscoll, 2015; Rhee, 2018).

Dyslipidemia is an essential part of the metabolic syndrome, in addition to abdominal obesity and insulin resistance (Vekic et al., 2019). We have previously demonstrated that the postprandial triglyceride response (TGR) is altered in healthy individuals with obesity, with a delayed postprandial triglyceride (TG) clearance compared to healthy, normal weight individuals (Larsen et al., 2015). Because humans spend most of their waking hours in the postprandial state, this increased period of triglyceride exposure in obese individuals contributes to an increased risk of both CVD and T2DM, by increasing atherosclerosis and induce insulin resistance (Tenenbaum et al., 2014; Huet et al., 2019).

The adipokines leptin and adiponectin are, together with free fatty acids (FFA), critical mediators in adipocytes to maintain metabolic homeostasis (Stern Jennifer et al., 2016; Harris, 2014; Wang and Scherer, 2016). Moreover, adipokines play a central role in modulating inflammation (Naylor and Petri, 2016; Unamuno et al., 2018), another contributing mechanism to CVD. In individuals with overweight and obesity, alterations of adipokine levels and their function are essential factors of the pathophysiologic mechanisms causing the metabolic syndrome, CVD, T2DM and other complications to obesity (Unamuno et al., 2018; Souza et al., 2017).

Abbreviations: ALT, Alanine aminotransferase; ANOVA, Analysis of variance; Apo, Apoliopoprotein (A1, B and E); AST, Aspartate aminotransferase; BMI, Body mass index; BP, Blood pressure; CI, Confidence interval; CVD, Cardiovascular disease; DEXA, Dual-energy X-ray absorptiometry; ELISA, Enzyme-linked immunosorbent assay; FFA, Free fatty acid; γ-GT, Gamma glutamyl transferase; HDL, High-density lipoprotein; HOMA-IR, Homeostasis model assessment of insulin resistance; L:A, Leptin to adiponectin (ratio); LDL, Low-density lipoprotein; OGTT, Oral glucose tolerance test; OFTT, Oral fat tolerance test; REE, Resting energy expenditure; REK, Regional etisk komité (Regional ethics committee); TG, Triglyceride; TGR, Triglyceride response; UiT, University of Tromsø; UNN, University hospital of North Norway; WBISI, Whole body insulin sensitivity index.

* Corresponding author at: Faculty of Health Sciences, UiT The Arctic University of Norway, Norway.
E-mail addresses: dr.isaksen@gmail.com (V.T. Isaksen), doctorarlen@gmail.com (M.A. Larsen), rasmus.goll@uit.no (R. Goll), eyvind.j.paulssen@uit.no (E.J. Paulssen), jon.florholmen@unn.no (J.R. Florholmen).
[1] Present address: Storbakkvegen 29, N-9020 Tromsdalen, Norway.

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Obesity is related to leptin resistance, a well-known concept first described in 2000 (Aizawa-Abe et al., 2000). Leptin levels are correlated to fat mass and thus signals energy balance (Elias and Purohit, 2013). Obese individuals are almost always hyperleptinemic while having a normal resting energy expenditure (REE), whereas normal weight individuals maintain a normal REE on lower leptin levels (Lustig et al., 2004). Reduced leptin levels signal energy deficiency, which in turn induces counter-regulatory responses, of which reduced REE is one (Elias and Purohit, 2013). Thus, the effect of leptin on REE is shown to be feasible as an indirect marker of leptin sensitivity, by using the REE to leptin (REE:leptin) ratio (Lustig et al., 2004).

Leptin and adiponectin changes are seen after a fatty meal in normal weight individuals, but not in obese individuals (Larsen et al., 2018). Furthermore, the leptin to adiponectin (L:A) ratio has been proven to correlate well with other aspects of metabolic dysregulation and risk of chronic metabolic disease (Li et al., 2017; Kang et al., 2017; Frithioff-Bøjsøe et al., 2020). Individuals with obesity and elevated L:A ratio tend to have a delayed TG clearance, as well as both insulin and leptin resistance, making the L:A ratio a useful surrogate marker for the metabolic syndrome (Larsen et al., 2018).

A modest weight loss of ≥5 % improves metabolic disturbances and clinical features of the metabolic syndrome and complications of T2DM, with the improvement of insulin sensitivity being a crucial element (Magkos et al., 2016; Clamp et al., 2017). Furthermore, because of compensatory metabolic mechanisms during weight loss, a modest weight loss might be easier to maintain in the long run than a > 10 % weight loss (Nymo et al., 2018). Improvement of central leptin satiety signalling, as expressed in feeding behaviour, with weight loss and reduced leptin levels have previously been demonstrated (Andreoli et al., 2019). However, to our knowledge, documentation of leptin resensitization has only been described in experimental animal weight loss models, not in humans (Andreoli et al., 2019).

Thus, this study aimed to investigate if a modest weight loss of ≥5 % is sufficient for substantial improvement and possibly normalization of the L:A ratio, as identified in our normal weight controls (Larsen et al., 2018), and if a corresponding improvement of other biomarkers of subclinical metabolic dysregulation occurs (Larsen et al., 2018; Ryan and Yockey, 2017).

2. Materials and methods

We recruited participants from the Centre for Obesity, Department of Gastroenterology and Nutrition, from the obesity rehabilitation program at Stamina Health Tromsø (later renamed Avonova) and by posters placed at the Department of Clinical Nutrition and Department of Endocrinology at the University Hospital of North Norway (UNN). Eligible patients were provided with oral and written information and signed a written consent to participate.

Inclusion of participants is shown in Fig. 1.

Inclusion criteria for the study population were a baseline body mass index (BMI) ≥30 kg/m^2 and age ≥ 18 years. Exclusion criteria were smoking, pregnancy, severe mental illness, previous heart disease, medically treated diabetes mellitus and kidney failure. We excluded patients who, for any reason, dropped out of the weight loss program. Height, body weight, blood pressure and pulse were measured. Total, abdominal and gynoid fat percentage, total fat mass (kg) and total muscle mass (kg) were obtained from body composition measured at baseline and follow-up after weight loss treatment, using Dual-Energy X-ray Absorptiometry (DEXA; Lunar Prodigy Advance, GE Health Care, USA).

2.1. Normal weight control group

For our control group we recruited 17 healthy, normal weight participants from the general population. Inclusion criteria were BMI in normal range, between 18 and 40 years of age, normotensive, normoglycemic, normolipemic and no history of diabetes. Exclusion criteria were otherwise the same as for our obese participants. The control participants underwent the same measurements and tests as our obese participants. Their results were used to create 95 % confidence intervals (CI) for normal values in our metabolic parameters.

2.2. Weight loss intervention

The weight loss goal for the participants with obesity was a minimum of ≥5 % of the baseline body weight, as this amount of weight loss is accepted as clinically meaningful (Ryan and Yockey, 2017). The post-weight loss tests were performed within two years after the weight loss intervention. A shortened follow-up period was selected for participants who lost weight quickly, in order to avoid loss to follow-up due to weight regain.

Weight loss intervention was performed either individually or in treatment groups in the programs from where participants were recruited. The intervention was based on Norwegian national guidelines for diet and exercise (Nordic Council of Ministers, 2014; Norwegian Directorate of Health, 2015). Since the study aimed to investigate the effects of weight loss *per se*, the instructions for weight loss methods were kept liberal and not formally recorded. Participants were free to adjust their diet and exercise according to their own preferences, within national guidelines. To avoid bias on outcome variables from differences in diet and exercise we included a three-day period with no strenuous physical exercise prior to visits and also asked participants to abstain from heavy meals and alcohol the day before visits.

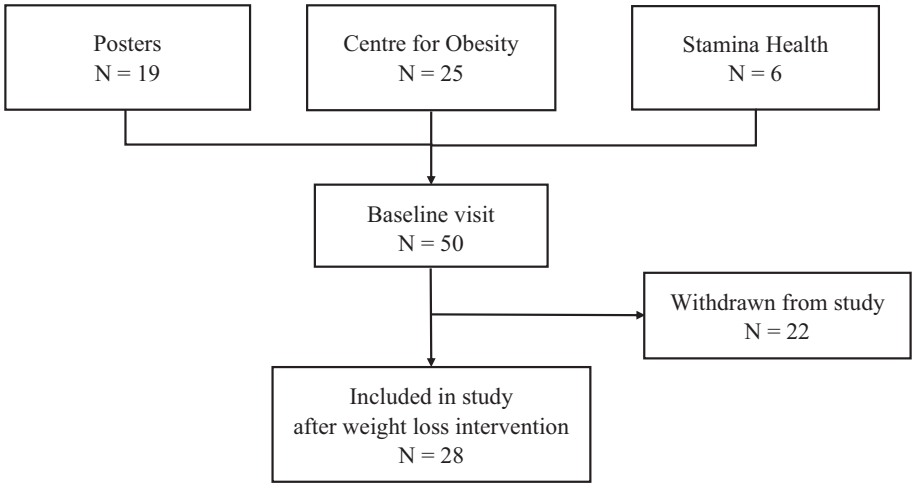

Fig. 1. Flowchart of included participants from posters at UNN, obesity out-patient clinic at UNN and Stamina obesity rehabilitation program, respectively.

All 50 participants who underwent baseline tests were offered to undergo the second round of tests regardless of the amount of weight loss during follow-up. Twenty two out of 37 participants in the non-weight loss group declined the offer and was hence lost to follow-up, while 15 accepted. All 13 participants in the weight loss group accepted the offer.

2.3. Insulin sensitivity

We performed a 2 hour (h) Oral Glucose Tolerance Test (OGTT) (Larsen et al., 2018). Participants had their regular diet and abstained from vigorous exercise three days before the test and showed up at 08:00 am after 12 h of overnight fasting. The test was conducted by oral intake of 75 g glucose dissolved in water (Matsuda and DeFronzo, 1999). We collected blood samples in both the fasting state and 30, 60, 90 and 120 min after glucose intake, in which serum (s-) glucose was measured by UV analysis (Cobas Integra Systems, Roche Diagnostics, Indianapolis, IN, USA) and s-insulin was measured using ELISA kits (DRG Insulin Elisa kit, DRG Instruments GmbH, Germany) (Larsen et al., 2015). We determined insulin sensitivity by calculation of the HOMA-IR (Matthews et al., 1985), as it has previously proven to correspond well to the whole body insulin sensitivity index (WBISI) (Matsuda and DeFronzo, 1999; Isaksen et al., 2016).

2.4. S-leptin and adiponectin measurements

Both fasting s-leptin and fasting free s-adiponectin were analysed from frozen serum drawn at all sample times, both during Oral Fat Tolerance Test (OFTT) and OGTT, using ELISA kits (DRG Diagnostics, Marburg, Germany) for leptin (sandwich ref. EIA-2395) and adiponectin (human, ref. EIA-4574), respectively. From these measurements, the L:A ratio was calculated as follows:

$$\text{L : A ratio} = \frac{\text{serum leptin}}{\text{serum adiponectin}}$$

As the intra individual variation was minimal between OFTT and OGTT measurements, OFTT values were selected for the statistical analyses.

2.5. Leptin sensitivity

Leptin sensitivity was calculated as the ratio of Resting Energy Expenditure (REE) to fasting s-leptin (Lustig et al., 2004; Bi et al., 2018). We performed REE measurements by a canopy test with an indirect calorimetry device from Medical Graphics CPX metabolic cart (St Paul, MN, USA).

The calorimetry was performed for 30 min in a supine position and in a resting and fasting state. REE was derived from the respiratory exchange ratio and the respiratory quotient (Larsen et al., 2018). After the completion of REE measurement, the OGTT was performed (Lustig et al., 2004).

2.6. Postprandial triglyceride clearance

To measure postprandial TGR and TG clearance, we performed an OFTT on a separate day from the OGTT (Cohen, 1989; Lekhal et al., 2008). Preparations for the OFTT were the same as for the OGTT. Fasting blood samples were drawn, and a meal of sour cream porridge (Fjordland sour cream porridge and full-fat cream in 1:1 ratio) was served. The meal contained 70 % calories of fat (66 % saturated fat, 32 % monounsaturated, 2 % polyunsaturated fat). The portions were adjusted to contain 1 g fat/kg body weight (Lekhal et al., 2008). The participants ingested the meal within 30 min, and blood samples were drawn from the antecubital vein in a seated position at baseline and 2, 4, 6 and 8 h postprandially.

The Department for Clinical Biochemistry at UNN analysed fasting serum lipids using a Hitachi 737 automatic analyser (Boehringer Mannheim GmbH, Mannheim, Germany) (Larsen et al., 2015).

The TGR was defined as the average of the two highest postprandial TG concentrations, minus the baseline concentration (Larsen et al., 2015; Lekhal et al., 2008).

The formula for calculating TG clearances (Lekhal et al., 2008) at time X was as follows:

$$\text{Clearance Xh} = 100 \times \left(1 - \frac{TG_X - TG_{0h}}{TG_{max} - TG_{0h}}\right)$$

2.7. Cut-off values for metabolic parameters

Normalization of metabolic parameters were defined as reaching the 95 % CI of our healthy, normal weight control group. The cut-off values were as follows: TG clearance at 6 h $\geq$ 88 %, TGR $\leq$ 0.67, HOMA-IR $\leq$ 1.83, WBISI $\geq$ 131.4, L:A ratio $\leq$ 1.19 and REE:leptin ratio $\geq$ 114.5 (Larsen et al., 2018).

2.8. Statistics

For statistical calculations, we used IBM SPSS Statistics 25 for Windows (SPSS Inc., Chicago, IL, USA). For calculating TG clearance and postprandial TGR, we used Microsoft Excel (Microsoft corp., Redmond, WA, USA). Plots were generated in GraphPad Prism 7 (GraphPad Software, La Jolla, CA, USA).

We used parametric tests on raw or transformed variables that resembled a normal distribution visually or by skewness/kurtosis for baseline analyses. Otherwise, Mann-Whitney non-parametric tests were performed for baseline analyses. To compare pre and post weight loss data we performed Related Samples Wilcoxon Rank Test. Due to small sample size complex analyses, such as ANOVA, were not possible to perform. Statistics for normal weight control group are previously described (Larsen et al., 2018).

3. Results

3.1. Baseline characteristics

We included 28 Caucasian participants in this study with a weight loss of 0–30 %. Seven participants (25 %) were male and 21 (75 %) were female. Among these, 15 participants (54 %) lost < 5 % body weight whereas 13 participants (46 %) lost $\geq$ 5 % body weight, including 5 participants (18 %) who lost $\geq$ 10 %. Median weight loss in the weight loss group was 10.0 kg.

Clinical, anthropometric, and metabolic characteristics are shown in Table 1. There were no significant differences in baseline characteristics between the weight loss and non-weight loss group, with the exception of REE ($p = 0.006$), TG ($p = 0.011$) and HDL-cholesterol ($p = 0.004$) levels. For leptin and adiponectin measurements, only data from OFTT samples are shown.

Four participants (three in the weight loss group, one in the non-weight loss group) used antihypertensive medication at baseline, two used thyroid replacement drugs (one in each group) and three used lipid lowering drugs (three in the weight loss and one in the non-weight loss group). Two participants in the weight loss group had hypertension at baseline. All female participants except one had LDL-cholesterol levels < 4.3 mmol/L, the upper limit of normal for females < 50 years. All male participants except one had LDL-cholesterol levels < 4.7 mmol/L, the upper limit of normal for males < 50 years.

Our normal weight controls had a median fasting s-leptin of 8.5 ng/mL and fasting free s-adiponectin of 11.8 μg/mL.

3.2. L:A ratio

There were significant improvements between pre- and post-intervention visits for fasting s-leptin and L:A ratio for the weight loss group, but not for the non- weight loss group (Table 2). Participants with weight loss had a 45.7 % ($p = 0.002$) improvement in L:A ratio (Table 2).

Fig. 2 shows the case-by-case change in central variables before and after weight loss intervention. Most notably is the significant improvement

Table 1
Baseline characteristics of the 28 overweight participants and 17 normal weight controls.

	Normal weight controls, $n = 17$	Weight loss ≥ 5 %, $n = 13$	Weight loss < 5 %, $n = 18$	Sig. (p)
Sex (M/F)	2/15	2/11	5/10	0.282
Age	31.0 (13)	39.8 (13)	36.0 (19)	0.254^{M}
BMI (kg/m^2)	21.3 (2.2)	33.6 (11.5)	39.8 (7.5)	0.217^{M}
Total body fat (%)	26.6 (5.9)	50.5 (9.5)	49.1 (9.1)	0.142^{M}
Abdominal fat (%)	27.5 (6.4)	58.2 (7.9)	56.7 (3.9)	0.895^{G}
Gynoid fat (%)	36.3 (5.8)	54.5 (9.1)	53.6 (9.8)	0.118^{M}
REE (kcal/day)	1356 (185)	1604 (441)	1989 (465)	0.006^{M}
Systolic blood pressure (mmHg)	105 (15)	124 (22)	128 (15)	0.525^{M}
Diastolic blood pressure (mmHg)	65 (10)	80 (17)	75 (9)	0.208^{G}
Fasting glucose (mmol/L)	4.4 (0.7)	5.4 (1.3)	5.1 (1.0)	0.892^{M}
Fasting insulin (μmol/L)	5.5 (2.9)	10.8 (6.2)	13.2 (6.1)	0.142^{M}
HOMA-IR	1.1 (0.7)	2.7 (1.8)	3.1 (1.9)	0.170^{M}
WBISI	147.8 (101.5)	55.1 (45.6)	55.2 (31.2)	0.467^{M}
Fasting TG (mmol/L)	1.0 (0.4)	1.2 (0.7)	1.5 (0.6)	0.011
TGR (mmol/L)	0.3 (0.3)	0.6 (0.4)	1.0 (0.9)	0.586^{M}
TG clearance 6 h (%)	115.4 (62.3)	65.0 (96)	61.1 (46)	0.413^{M}
Fasting leptin (ng/mL)	8.5 (7.4)	32.5 (37.0)	41.7 (21.8)	0.928^{M}
Fasting adiponectin (μg/mL)	11.8 (7.1)	9.0 (3.7)	6.8 (3.7)	0.525^{M}
L:A ratio	0.6 (0,9)	3.8 (2.5)	6.1 (35.2)	0.683^{M}
REE:Leptin ratio	142.5 (134.0)	54.1 (51.6)	47.8 (51.6)	0.683^{M}
Total cholesterol (mmol/L)	4.2 (0.9)	4.3 (1.2)	4.7 (1.1)	0.153^{G}
LDL cholesterol (mmol/L)	2.6 (1.3)	2.7 (1.4)	2.9 (0.9)	0.107
HDL cholesterol (mmol/L)	1.6 (0.5)	1.3 (0.2)	1.1 (0.2)	0.004
HDL:LDL ratio	0.57 (0.49)	0.52 (0.47)	0.35 (0.17)	0.009^{M}

Baseline anthropometric and metabolic characteristics for all participants. Significance tested between weight loss groups by *t*-test or Mann-Whitney non-parametric test (^{M}). Parameters without normal variation distribution were transformed to geometric mean (^{G}) if possible before the t-test was performed. Values shown as median (interquartile range). Abbreviations: BMI Body Mass Index. HDL High Density Lipoprotein. HOMA-IR Homeostasis Model Assessment of Insulin Resistance. L:A ratio Leptin:Adiponectin ratio. LDL Low Density Lipoprotein. REE Resting Energy Expenditure. TG Triglyceride. TGR Triglyceride Response. WBISI Whole Body Insulin Sensitivity Index.

in L:A ratio in participants in the weight loss group. The improvement was even greater in the subgroup of ≥10 % weight loss, but not reaching the level of normality (cut-off value ≥ 1.88, $p = 0.030$, Fig. 2). In the non-weight loss group no significant changes were observed ($p = 0.020$).

3.3. Leptin sensitivity

The REE:leptin ratio improved with 80.1 % ($p = 0.005$) in the weight loss group (Table 2) but not in the non-weight loss group. Furthermore, there was a tendency of greater improvements in leptin sensitivity in the subgroup of ≥10 % weight loss, compared to 5–10 % weight loss. Four of the 13 (31 %) participants in the subgroup achieved normalized leptin sensitivity after weight loss (REE:leptin ratio ≥ 114.5, Fig. 2). No significant changes were observed in the non-weight loss group ($p = 0.013$).

3.4. Insulin sensitivity

Insulin sensitivity measured by HOMA-IR improved with 23.1 % ($p = 0.011$) in the weight loss group, where nine out of the 13 (69 %)

Table 2
Post-intervention characteristics for the 28 participants.

	Weight loss ≥ 5 %			Weight loss < 5 %		
	Value	Per cent change	Sig. (p)^{W}	Value	Per cent change	Sig. (p)^{W}
Weight (kg)	81.1 (24.1)	−10.0 (7.1)	0.001	119.9 (18.8)	−1.1 (4.7)	0.691
BMI (kg/m^2)	30.9 (8.9)	−8.2 (6.2)	0.001	39.5 (7.1)	−1.9 (4.3)	0.379
Abdominal fat (%)	51.1 (7.6)	−7.4 (7.6)	0.001	55.6 (6.5)	0.0 (9.5)	0.917
Gynoid fat (%)	52.0 (11.3)	−5.4 (8.4)	0.002	51.2 (13.5)	−0.6 (6.5)	0.778
HOMA-IR	1.4 (1.8)	−23.1 (51.6)	0.011	2.6 (1.3)	−6.8 (52.6)	0.807
WBISI	92.1 (163.2)	49.9 (133.8)	0.008	67.2 (30.0)	13.1 (50.4)	0.477
L:A ratio	2.7 (2.2)	−45.7 (29.9)	0.002	5.0 (4.7)	−6.6 (26.8)	0.196
REE:Leptin ratio	78.5 (72.9)	80.1 (92.6)	0.005	71.3 (68.0)	0.9 (45.8)	0.480
REE (kcal/day)	1459 (427)	−8.6 (15.6)	0.196	2095 (360)	0.0 (10.4)	0.600
Fasting TG (mmol/L)	1.1 (0.6)	−9.2 (66.0)	0.649	1.3 (1.2)	−13.3 (49.7)	0.507
TGR (mmol/L)	0.51 (0.59)	−33.6 (29.6)	0.327	0.67 (1.03)	−24.4 (148.8)	0.754
TG clearance 6 h (%)	75 (62.1)	6.6 (116.6)	0.807	66.0 (42.1)	−7.1 (453.3)	0.778
Systolic blood pressure (mmHg)	121 (18)	0.0 (8.4)	0.074	120 (15)	−6.3 (15.9)	0.020
Diastolic blood pressure (mmHg)	76 (20)	0.6 (12.9)	0.248	69 (16)	1.5 (26.4)	0.409
Fasting leptin (ng/mL)	20.5 (14.3)	−50.0 (36.3)	0.004	29.8 (27.8)	−3.4 (15.2)	0.814
Fasting adiponectin (μg/mL)	8.2 (4.6)	−4.1 (42.5)	0.576	7.1 (2.4)	11.0 (19.5)	0.433
Fasting glucose (mmol/L)	5.0 (1.1)	−5.3 (11.8)	0.090	5.1 (1.6)	−2.1 (12.3)	0.339
Fasting insulin (μmol/L)	6.3 (6.6)	−16.6 (45.2)	0.006	8.3 (8.4)	−4.6 (56.6)	0.470
LDL cholesterol (mmol/L)	2.8 (1.0)	−11.1 (34.9)	0.438	4.8 (1.0)	−2.9 (32.3)	0.972
HDL cholesterol (mmol/L)	1.4 (0.3)	−0.2 (19.3)	0.872	3.0 (0.8)	10.0 (21.2)	0.715

Post-intervention characteristics and per cent change from baseline characteristics for weight loss (≥5 %) and non-weight loss (<5 %) groups, respectively. Significance tested between baseline and post-treatment values by Related Samples Wilcoxon Rank Test (^{W}). Values shown as median (interquartile range). Abbreviations: BMI Body Mass Index. HDL High Density Lipoprotein. HOMA-IR Homeostasis Model Assessment of Insulin Resistance. L:A ratio Leptin:Adiponectin ratio. LDL Low Density Lipoprotein. REE Resting Energy Expenditure. TG Triglyceride. TGR Triglyceride Response. WBISI Whole Body Insulin Sensitivity Index.

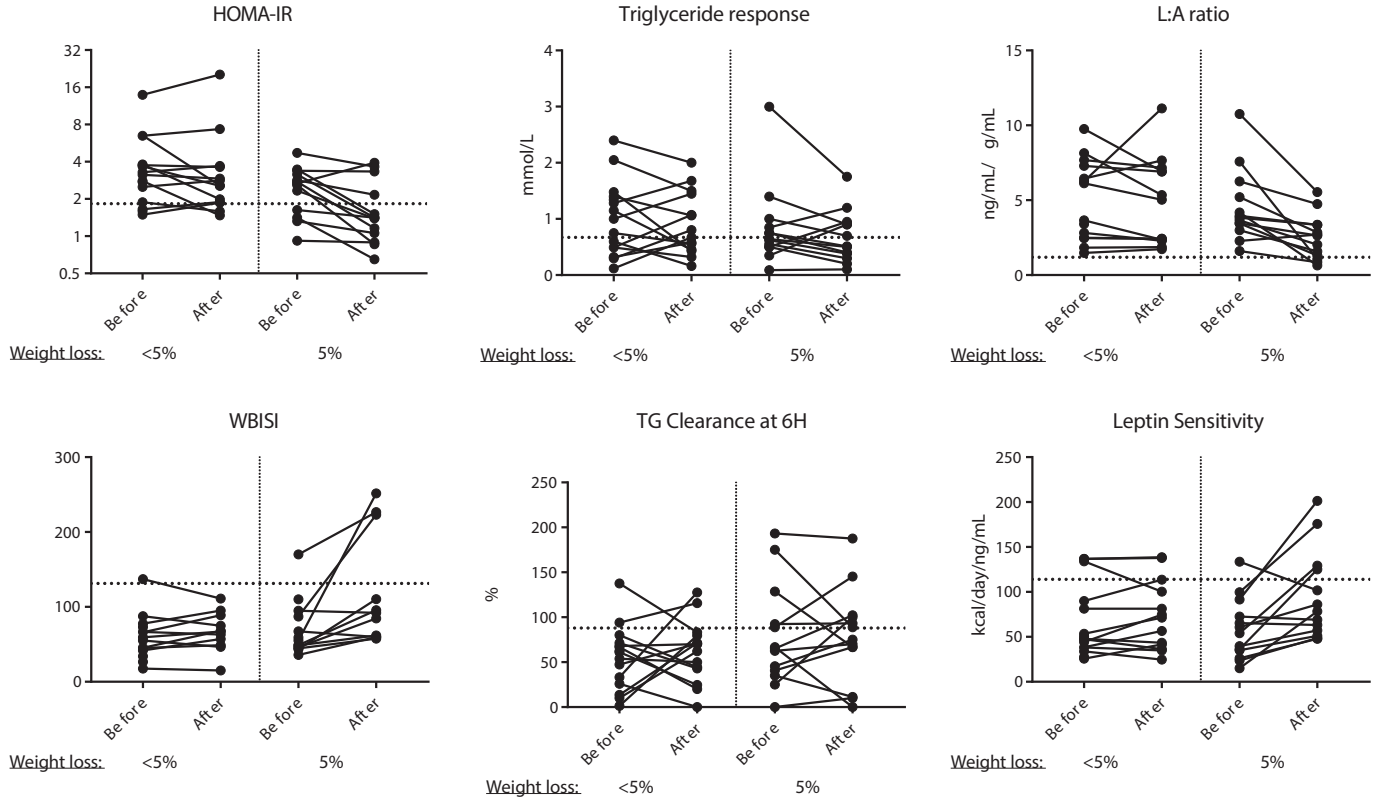

Fig. 2. Difference between baseline and post intervention values of A) L:A ratio, B) Indirect leptin sensitivity (REE:Leptin ratio) C) HOMA-IR, D) TG clearance at 6 h and E) TGR before and after <5 % and ≥ 5 % weight loss in individual participants. Horizontal lines represent the upper or lower limit of 95 % CI in healthy, normal weight controls.

participants had normal HOMA-IR values after weight loss (HOMA-IR ≤ 1.83) Of these, five participants improved from insulin resistance at baseline.

There also was a significant improvement of 49.9 % in WBISI in the weight loss group ($p = 0.008$). Two participants in this group normalized their WBISI, while one participant maintained a normal baseline WBISI. There also was a significant difference in WBISI delta values between weight loss and non-weight loss group (p = 0.013, Mann-Whitney *U* test).

No significant changes were observed in the non-weight loss group for neither HOMA-IR nor WBISI (Table 2, Fig. 2).

3.5. Improvement of postprandial triglycerides

No significant differences in postprandial TG clearance at 6 h were seen in any of the groups (6.6 %, $p = 0.807$). (Table 2, Fig. 2).

4. Discussion

In this study we have examined the L:A ratio, indirect leptin sensitivity, insulin sensitivity, and postprandial TG metabolism in obese participants before and after a modest weight loss of ≥5 %. We found significant improvements in L:A ratio (−45.7 %), leptin sensitivity (−80.1 %) and insulin sensitivity (−23.1 %) in participants who achieved weight loss, compared to participants with no weight loss. Furthermore, our study shows that as little as ≥5 % weight loss improves adipokines, but not TG metabolism.

To our knowledge, few studies have been performed to assess L:A ratio, insulin sensitivity and REE:leptin ratio in a population of otherwise largely healthy adult individuals undergoing long term lifestyle intervention to achieve weight loss. However, Ho et al. found improved insulin sensitivity and L:A ratio after a 12 month lifestyle intervention for >10 % weight loss in obese healthy adults (Ho et al., 2015) and Miller et al. found significant improved leptin and adiponectin after a one-year lifestyle intervention for >5 % weight loss in obese healthy individuals (Miller et al., 2014). Both studies also found improvements in inflammation markers. Furthermore, our results are in line with studies reporting similar improvements in leptin and adiponectin levels with weight loss in more selective groups of children/adolescents (Alaby Martins Ferreira et al., 2020), type 1 diabetes patients (Musil et al., 2015), cancer patients (Befort et al., 2020), elderly (Ilich et al., 2022; Miller et al., 2012) and patients undergoing bariatric surgery (Farias et al., 2020), weight loss >20 % (Hausmann et al., 2019), or short-term, intensive weight loss programs (Moro et al., 2016; Kelly et al., 2014). Our findings are also in line with the findings of improved adiponectin levels and cardiometabolic risk in the Look AHEAD study on T2DM patients (Belalcazar et al., 2015; Group LAR and Gregg, 2016).

We have previously reported that the L:A ratio is a feasible surrogate biomarker for early detection of metabolic disturbances in obesity (Kang et al., 2017). Moreover, we have previously reported a potential postprandial regulatory role of adiponectin and leptin which is impaired in obesity (Larsen et al., 2018). The L:A ratio has been demonstrated to improve after a weight loss of 5–10 % by Ferreira et al. and Talaei et al. among others (Alaby Martins Ferreira et al., 2020; Talaei et al., 2017).

Our present findings demonstrate an improvement in L:A ratio of −45.7 % in a small group of 13 participants with weight loss. Such a large improvement in this small dataset has major clinical significance, and supports a realistic weight loss goal that is potentially easier to maintain long term (Nymo et al., 2018). The improvement of the L:A ratio is important as it is known to correlate to low-grade inflammation and thus risk of CVD (Alaby Martins Ferreira et al., 2020; Frühbeck et al., 2018; Frühbeck et al., 2019).

Frühbeck et al. proposed adiponectin:leptin ratio cardiovascular risk category limits as follows: normal risk ≥ 1, moderate risk 0.5–1 and high risk < 0.5 (Frühbeck et al., 2019). Inversely, this translates to our L:A ratio category limits of ≤1, 1–2 and >2, respectively. According to these limits of risk, three participants with weight loss crossed from high to moderate cardiovascular risk after weight loss while one participant crossed

from moderate to low risk and one from high to low risk. In total 38 % of the participants reduced their risk category.

A study by Bi et al. suggests that leptin contributes significantly more to the variance in REE than what is explained by fat mass (Bi et al., 2018). Furthermore, Rosenbaum et al. demonstrated that leptin administration reversed the decrease in energy expenditure after weight loss (Rosenbaum et al., 2002). We found that REE:leptin ratio improved with a median of 80.1 %. In addition, approximately one third (31 %) of the participants in the weight loss group had their REE:leptin ratio normalized after weight loss (Larsen et al., 2018). Although resensitization of leptin on satiety signalling is not possible to measure clinically in humans, one can speculate whether this demonstration of indirect resensitization of leptin suggests a normalization of central leptin sensitivity as well.

Insulin sensitivity, as measured by the HOMA-IR or WBISI, improved by 23.1 % and 49.9 %, respectively, after weight loss. Moreover, five out of the 13 (39 %) participants obtained normalized insulin sensitivity as defined by HOMA-IR, while two out of 13 (13 %) normalized it as defined by WBISI (Larsen et al., 2018; Bi et al., 2018). This is in agreement with most other reports (Clamp et al., 2017). The improved insulin sensitivity observed together with an improved L:A ratio is also in agreement with previous reports, describing a correlation between the two variables (Frithioff-Bøjsøe et al., 2020). Our findings of a substantial percentage of normalized insulin sensitivity further support the conclusion that a 5 % weight loss is clinically meaningful for reducing obesity complications.

There was no significant improvement of postprandial TG clearance after ≥5 % weight loss. This could be explained by the small number of participants that lost ≥ 10 % body weight. Magkos et al. reported that improvements of TG and FFA were observed first after 11 % and 16 % weight loss, respectively (Magkos et al., 2016). Therefore, normalization of postprandial TG clearance will most likely be observed only after a substantial weight loss in obese individuals.

The strengths of the present study are the use of several different methods, particularly adipokine levels, reflecting the pathophysiological mechanisms behind metabolic disorders in obesity to make comprehensive explanations of the results obtained by a modest weight loss. In addition to this, the generalizability of the weight loss method used in the study is high, as we did not assign one specific diet or exercise plan to our participants. We also included participants from different sources with broad inclusion criteria. The study was performed in a clinical setting, which also makes the generalizability high.

There are several weaknesses of this study. The main weakness of our study design is the high dropout rate, combined with a small sample size that renders us unable to perform parametric tests and to perform more complex analyses (ANOVA, *etc.*). The final sample size was way below our estimated sample size of 50 participants, and was explained by a large dropout rate. Due to the small sample size, we mainly used repeated measures analyses, thus reducing the variability in our samples, making our results more reliable under the given circumstances.

There is also a significant imbalance between men and women included in our study. This could affect our results, as body composition and hence leptin concentrations differ between the sexes (Ruhl et al., 2007). However, the gender difference in our study is in accordance with other studies of obesity, and improvements in adipokines with weight loss seem similar in studies on males and predominantly female participants (Miller et al., 2014; Borel et al., 2017). Furthermore, our study is in line with literature on differences in the population of patients seeking help for health problems in general (Galdas et al., 2005).

There could be possible confounding factors related to the geographical location of our study, above the Arctic Circle. These would include low levels of vitamin D, which is shown to be related to obesity, hyperleptinemia and insulin resistance (Madhu et al., 2022) and circadian rhythm disruptions due to differences in daylight exposure throughout the year (Noh, 2018). One could also mention low outside temperatures, although the evidence for its' relation to obesity is contradictory, and so will not be discussed further (Speakman and Heidari-Bakavoli, 2016).

We did not formally record these factors in our study, as they are beyond the scope of this paper. There is however a general advice to the Norwegian population to take vitamin D supplements during the winter months (Health NDo, 2014). Individuals who comply with this advice usually do so every year. Our participants were not prescribed any vitamin D supplements as part of their treatment at the obesity out-patient clinic beyond this. Furthermore, people who struggle to sleep during the summer months with midnight sun usually make sure to have dark blinders on their windows to reduce light exposure while they sleep.

As our participants served as their own controls in this study, we will argue that both vitamin D levels and nocturnal light exposure are sufficiently controlled for and would not significantly hinder our results' generalizability.

4.1. Conclusion

A modest weight loss of ≥5 % improves sensitive metabolic surrogate markers like L:A ratio, leptin sensitivity (REE:leptin ratio) and insulin sensitivity. However, markers of postprandial TG clearance did not improve at the present level of weight loss. Our results support that a realistic and achievable weight loss goal of ≥5 % for obese individuals does reduce the risk of metabolic complications. The findings in this study need to be confirmed and further evaluated in a larger study population.

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Ethics

All participants signed a written consent form. The Regional Ethics Committee of North Norway (2011/1677/REK Nord) approved the study. Consent for publication of results was not applicable, as no individual participant is identifiable in this paper.

Credit authorship contribution statement

Authors VTI and MAL conducted participant inclusion and data collection. MAL performed weight loss follow-up for part of the study group. VTI and RG performed statistical analyses. JF was the principal investigator.

All authors participated in the planning of the study, the interpretation of results and the process of writing and editing the manuscript.

Data availability

The dataset supporting the conclusions of this article is available in the UiT Open Research Data repository at https://doi.org/10.18710/KRYLXN

Declaration of competing interest

None.

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