# Anti-ApoA-1 predicts resistance to waist circumference reduction after Mediterranean Diet

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**Running title:** AntiApoA-1 IGs and waist circumference changes after Mediterranean diet


**Key words:** anti-ApoA-1 antibodies, waist circumference, metabolic syndrome, body mass index, inflammation, Mediterranean diet.

# 1. Introduction

The prevalence of obesity has doubled in the last half-century and continues to rise globally. However, a clear definition of this pathological condition, based on standardized clinically relevant variables, is still missing. Even the body mass index (BMI), which has been used for decades, has never emerged over traditional risk factors. Rather, the fall of BMI has contributed to reveal the heterogeneity of obesity [1]. Indeed, patients with similar BMI values – and body weight also – display different metabolic profiles and consequent cardiovascular (CV) risk. Body fat distribution and dysfunction (also referred to as "adiposopathy") has then become the new paradigm in obesity heterogeneity [1-3]. The shift towards visceral and ectopic fat distribution may be then better described by other anthropometric measures such as waist circumference (WC) and waist-to-hip ratio, alongside with direct imaging-based measurement of adipose tissue depots. Despite being clinically easy to implement, WC alone is only slightly stronger associated with metabolic outcomes than BMI and also the correlation with imaging-assessed visceral adiposity is modest. Even more relevant, validated predictors of neither WC/weight loss nor metabolic improvement in patients undergoing bariatric surgery or dietary interventions are still lacking. In this regard, a multitude of pathways involving genetic background, lifestyle, environmental pollution and especially low-grade chronic inflammation is under investigation [4-6]. Here, we attempted to deepen knowledge on the potential metabolic role of autoantibodies against apolipoprotein A-1 (anti-apoApoA-1 IgG), the major proteic fraction of high-density lipoprotein cholesterol (HDL-c). Detected at high levels in about 20% of general population, these antibodies represent an independent CV risk factor predictive of incident CV events [7] associated with atherosclerotic plaque vulnerability and a pro-inflammatory systemic profile. Several reports indicate that anti-ApoA-1 IgG are

associated with a more favorable lipid profile despite promoting foam cell formation [8,9]. Somehow counterintuitive, this observation has been mechanistically related to the propensity of these antibodies to re-orient intracellular towards cellular pools by the concomitant inhibition of cholesterol efflux and acetyl-CoA acetyltransferase 1 (ACAT) activity stimulation [8,9]. Being ACAT-1 activity causally involved in diet-induced obesity in vivo, selective ACAT-1 inhibition is currently being considered as a therapeutic mean to obesity and metabolic syndrome (MetS). Therefore, we hypothesized that the presence of anti-apoA-1 IgG could associate with a reduction in waist circumference during caloric intake restriction. To challenge this hypothesis, we measured circulating levels of anti-ApoA-1 IgG a cohort of outward patients undergoing a Mediterranean diet (MD) intervention for 1 year and assessed the relationship between these antibodies with WC loss (and related features) at 1-year follow-up after the intervention.

## 2.    Material and methods

### 2.1. Patients

This is a sub-analysis of a longitudinal observational monocentric study enrolling 141 consecutive patients aged 30 to 65 years referred for one or more diagnostic criteria for MetS to the Division of Internal Medicine Clinica Medica "A. Murri", Department of Biomedical Sciences & Human Oncology, University of Bari Medical School (Italy). Exclusion criteria have been previously reported [10]. For this pilot study, only subjects with increased WC, defined by a WC ≥96 cm in men and ≥80 cm in women, as recommended for Mediterranean/European population, were included [11]. The cohort includes all patients who completed one year of follow-up (n=49). During the visit at the outpatient clinic at baseline and one year after, the adherence to the MD-based

was assessed based on a eighteen-point scale [12]. Adherence was then defined as "inadequate" by a score of less than 10. Ongoing medical therapies were maintained, whereas treatments for newly diagnosed metabolic disease were standardized for all patients. In addition, all patients received dietetic, psychological and physical activity counseling and were treated for other comorbidities according to current guidelines [6]. The Ethics Committee of University Hospital Policlinico in Bari (Italy) approved this protocol, performed in accordance to the guidelines of the Declaration of Helsinki (study number 5408, protocol number 0013869; approved by AOUCPG23/COMET/P on 7th July 2017). Patients provided full informed consent before initiating the study.

## 2.2. Study endpoints

The primary outcome of this pilot study was to determine whether anti-ApoA-1 IgGs predict WC reduction during follow-up. Changes in WC were categorized as stable/increase vs. reduction. Null hypothesis was rejected for a type I error <0.05. Based on existing literature – and taking into account an expected prevalence of anti-ApoA-1 IgG of 20% - a sample size of 81 patients was requested to observe an OR of 1.5 with a power of 0.8. As secondary endpoints, we explored any correlation between anti-ApoA-1 IgG and parameters of metabolic dysfunction as well as potential determinants of WC loss in patients undergoing supervised lifestyle intervention, including as delta (Δ) weight-loss, Δ BMI loss, (Δ), epicardial ectopic fat loss and Δ visceral fat thickness during follow-up.

## 1.1. Data collection and assessment and anti-ApoA-1 IgG assay

For all patients, clinical data, including comorbidities and anthropometric measures, were recorded at baseline and after one year of follow-up. Anti-ApoA-1 IgGs were

measured as previously described [8,9]. Those methods are reported in the supplementary material.

### 1.2. Statistical analysis

Analyses were performed with IBM SPSS Statistics for Windows, Version 23.0 (IBM CO., Armonk, NY). Statistical methods are explained in the supplementary material.

## 2. Results

### 3.1. Patients distribution across clinical and biochemical parameters

The characteristics of the overall cohort appear in Supplementary Table 1. Median age of patients was 56 years with a slight prevalence of women (male 65.3%). Concerning MetS criteria, hypertension was highly frequent (83.7%) and BMI ranged from overweight to obese patterns (IQR 26.7–31.3 Kg/m$^2$). More than half of patients had one or two defining criteria for MetS, which was then observed in the 44.9% of patients. In line with the general population, the prevalence of anti-ApoA-1 IgG positivity was about 20% (18.4%), with a median optical density (OD) of 0.42 (0.31-0.60). Finally, median values of hs-CRP were 1.97 µg/mL (IQR 0.79-5.47).

When categorized for WC reduction during follow-up, patients experiencing good outcome showed a better adherence to MD, although this was only sub-optimal (median score still <10) (Table 1).

5

**Table 1. Clinical characteristics at baseline according with waist circumference reduction (n=26) or not (n=23).**

| | Waist circumference =/↑ (n=23) | Waist circumference ↓ (n=26) | *p*-value |
|---|---|---|---|
| **Clinical data** | | | |
| Age, yr. [IQR] | 54 [43-60] | 56 [45-61] | 0.880 |
| Males, n. (%) | 8 (34.8) | 9 (34.6) | 1.000 |
| Hypertension, no (%) | 17 (73.9) | 24 (92.3) | 0.125 |
| Type 2 diabetes, no. (%) | 5 (21.7) | 2 (7.7) | 0.230 |
| Active smokers, no. (%) | 2 (8.7) | 8 (30.8) | 0.080 |
| Systolic BP, mmHg [IQR] | 125 [118-135] | 130 [120-136] | 0.369 |
| Diastolic BP, mmHg [IQR] | 76 [70-82] | 80 [79-85] | 0.081 |
| Waist circumference, cm [IQR] | 103 [96-111] | 103 [97-120] | 0.912 |
| Weight, Kg [IQR] | 86 [69-94] | 81 [70-91] | 0.561 |
| BMI, Kg/m$^2$ [IQR] | 29.7.2 [26.9-32.5] | 29.1 [26.6-30.9] | 0.541 |
| Adherence MD score [IQR] | 7 [6-8] | 8 [7-9] | **0.009** |
| MetS criteria | | | 0.336 |
| 1, n. (%) | 3 (13.0) | 6 (16.2) | |
| 2, n. (%) | 10 (43.5) | 18 (48.6) | |
| 3, n. (%) | 8 (34.8) | 9 (34.6) | |
| 4, n. (%) | 2 (8.7) | 2 (7.7) | |
| 5, n. (%) | 0 (0.0) | 1 (3.8) | |
| MetS, no. (%) | 10 (43.5) | 12 (46.2) | 1.000 |
| **Ultrasound** | | | |
| EFth, mm [IQR] | 5.5 [4.7-6.5] | 6.0 [5.2-7.5] | 0.167 |
| VFth, mm [IQR] | 60 [46-72] | 64 [48-86] | 0.423 |
| **Chemistry** | | | |
| Serum total-c, mg/dl [IQR] | 199 [160-221] | 193 [168-223] | 0.638 |
| Serum LDL-c, mg/dl [IQR] | 108 [80-120] | 119 [91-145] | 0.128 |
| Serum HDL-c, mg/dl [IQR] | 68 [53-74] | 51 [46-63] | **0.016** |
| Serum TAG, mg/dl [IQR] | 97 [72-177] | 122 [94-158] | 0.383 |
| Fasting glycaemia, mg/dl [IQR] | 92 [72-177] | 122 [94-158] | 0.233 |
| VAI [IQR] | 1.2 [0.8-2.5] | 1.7 [1.4-2.4] | 0.118 |
| **Inflammatory biomarker** | | | |
| Anti-ApoA-I Ab, OD [IQR] | 0.58 [0.35-0.80] | 0.36 [0.28-0.48] | **0.003** |
| hs-CRP, µg/mL [IQR] | 2.32 [1.12-5.04] | 3.08 [0.90-6.08] | 0.548 |

Data are expressed as median (interquartile range [IQR]) or number [no.] (percentages [%]). Comparisons were drawn by Mann-Whitney U-test.

BP: blood pressure; BMI: body mass index; MD: Mediterranean Diet; MetS: metabolic syndrome; VFth: visceral fat thickness; EFth: epicardial fat thickness; cIMT: carotid intima media thickness; total-c: total cholesterol; LDL-c: low-density lipoprotein cholesterol; HDL-c: high-density lipoprotein cholesterol; TAG: triglyceride; VAI: visceral adiposity index; Anti-ApoA-1 Ab: anti-apolipoprotein A-I antibodies; hs-CRP: high-sensitivity C-reactive protein.

No differences were instead observed for biochemical parameters, except for circulating levels of HDL and anti-ApoA-1 antibodies ($p$=0.016 and 0.003, respectively).

*3.2.  Circulating levels of anti-ApoA-1 IgG are independently associated with poor response to therapy without affecting metabolic status at baseline.*

At baseline, circulating levels of anti-ApoA-1 IgG did not correlate with clinical or biochemical parameters, except for an inverse association with low-density lipoprotein cholesterol (LDL-c) levels ($r$=-0.331; $p$=0.020) (Table 2).

**Table 2. Correlation between anti-apolipoproteinA-1 antibodies (Anti-ApoA-1 Ab) and clinical parameters in the overall cohort at baseline.**
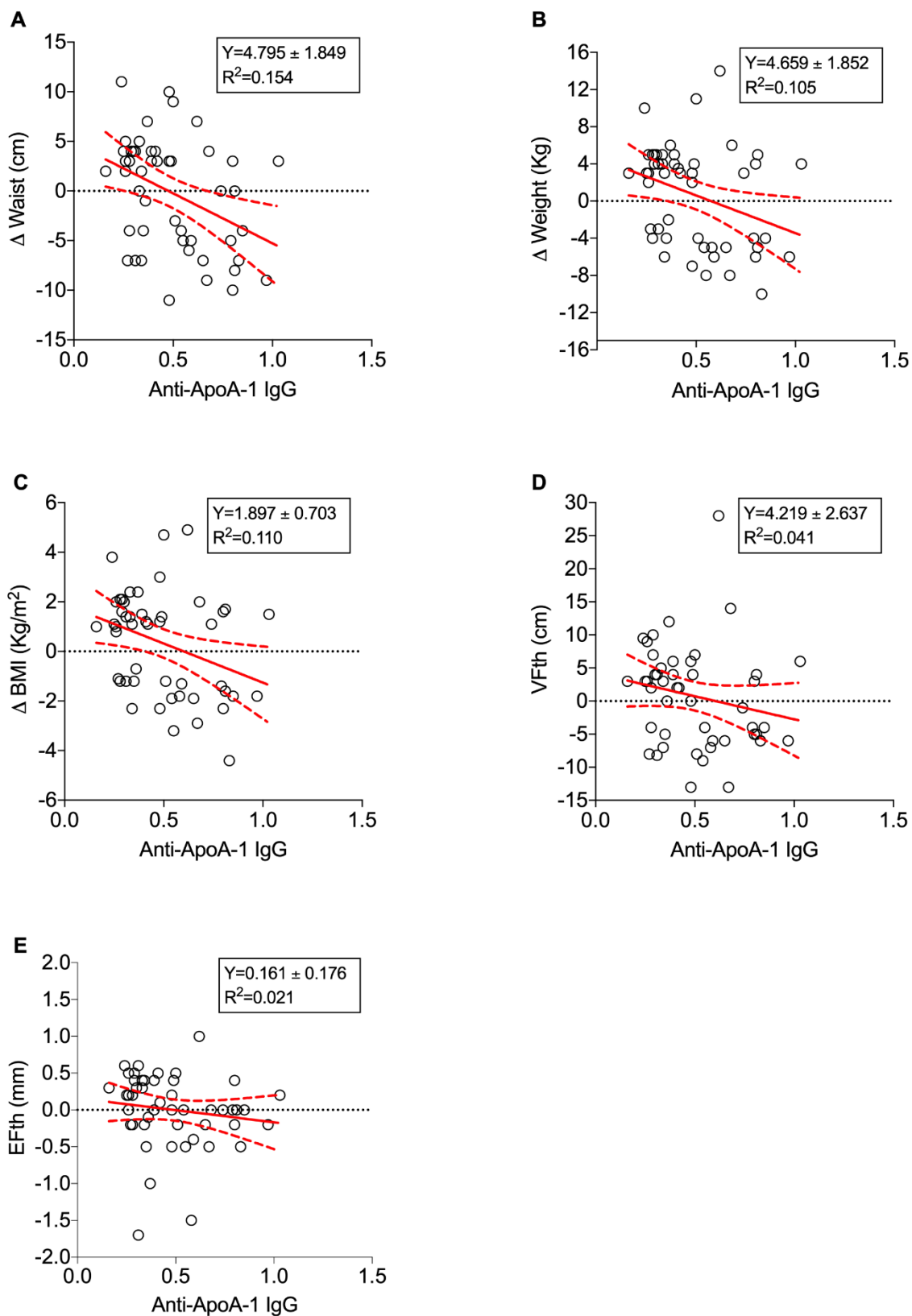
| Anti-ApoA-1 Ab | *r* | *p*-value |
|---|---|---|
| **Clinical parameters** | | |
| Age | -0.166 | 0.253 |
| sBP | 0.062 | 0.670 |
| dBP | -0.096 | 0.513 |
| Waist circumference | 0.001 | 0.993 |
| Weight | 0.088 | 0.549 |
| BMI | 0.061 | 0.679 |
| **Ultrasound** | | |
| VFth | -0.198 | 0.172 |
| EFth | 0.141 | 0.333 |
| **Chemistry** | | |
| Serum total-c | -0.255 | 0.077 |
| Serum LDL-c | -0.331 | **0.020** |
| Serum HDL-c | 0.016 | 0.914 |
| Serum TAG | -0.069 | 0.637 |
| Fasting glycaemia | 0.142 | 0.330 |
| VAI | -0.090 | 0.537 |
| **Inflammatory biomarker** | | |
| hs-CRP | 0.001 | 0.993 |

Comparisons were performed by Spearman's Rank correlation

BP: blood pressure; BMI: body mass index; VFth: visceral fat thickness; EFth: epicardial fat thickness; cIMT: carotid intima media thickness; total-c: total cholesterol; LDL-c: low-density lipoprotein cholesterol; HDL-c: high-density lipoprotein cholesterol; TAG: triglyceride; VAI: visceral adiposity index; hs-CRP: high-sensitivity C-reactive protein.

In line, there was no association with VAI ($r$=-0.090; $p$=0.537). Concerning categorical variables, only hypertension was associated with a higher anti-ApoA-1 IgG levels ($p$=0.013) (Supplementary Table 2). At prospective analysis, anti-ApoA-1 IgG levels at baseline correlated inversely with the reduction of adipose tissue depots during follow-up as assessed by Δ WC, Δ weight, Δ BMI, Δ epicardial ectopic fat depot, and partially Δ visceral fat thickness (Supplementary Table 3). Those correlations were also confirmed at linear regression analysis for Δ WC ($R2$=0.154; $p$=0.005), Δ weight ($R2$=0.105; $p$=0.023), and Δ BMI ($R2$=0.11; $p$=0.020) (Figure 1).

# Figure 1

**A**

$$Y=4.795 \pm 1.849$$
$$R^2=0.154$$

Δ Waist (cm) vs Anti-ApoA-1 IgG

**B**

$$Y=4.659 \pm 1.852$$
$$R^2=0.105$$

Δ Weight (Kg) vs Anti-ApoA-1 IgG

**C**

$$Y=1.897 \pm 0.703$$
$$R^2=0.110$$

Δ BMI (Kg/m$^2$) vs Anti-ApoA-1 IgG

**D**

$$Y=4.219 \pm 2.637$$
$$R^2=0.041$$

VFth (cm) vs Anti-ApoA-1 IgG

**E**

$$Y=0.161 \pm 0.176$$
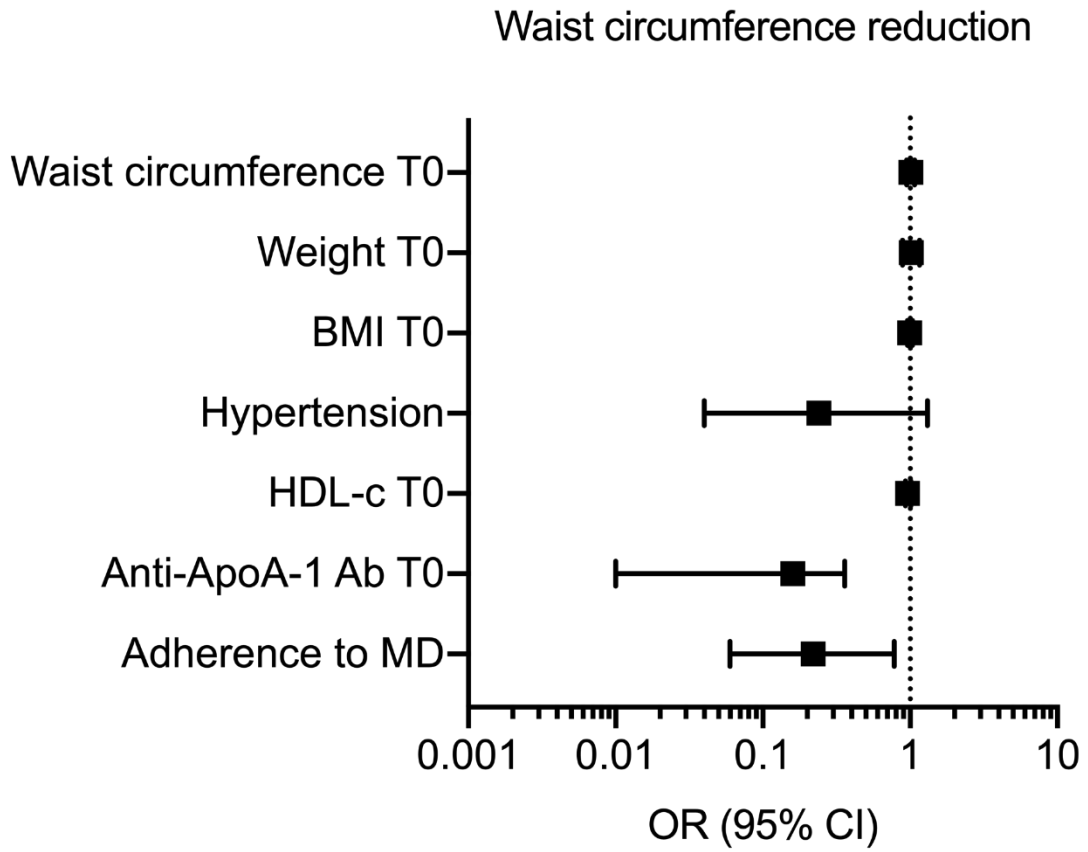$$R^2=0.021$$

EFth (mm) vs Anti-ApoA-1 IgG

When categorized as anti-ApoA-1 IgG positive/negative, anti-ApoA-1 IgG expression was characterized by a lower reduction of fat depots during follow-up. However, only for Δ waist this association reached the statistical significance ($p$=0.028) (Figure 2 and Supplementary Figure 1).

# Figure 2

**A**



**B**



**C**



**D**



**E**
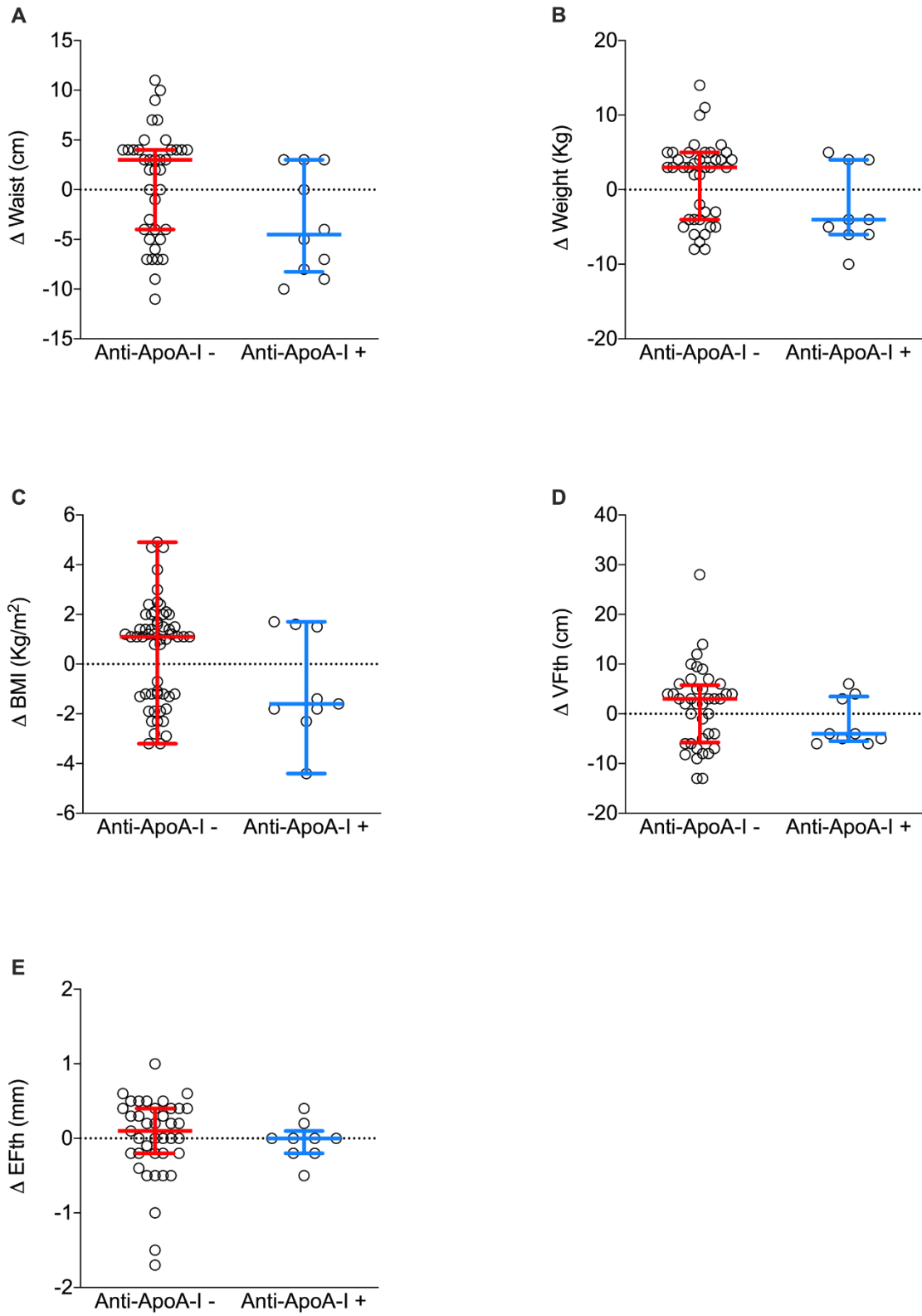
Finally, univariate logistic regression analyses (Figure 3 and Supplementary Table 4) indicated as significant predictors of poor WC reduction both the low adherence score to MD (OR 0.22 [95%CI 0.06-0.78]; $p$=0.049) and high circulating levels of anti-ApoA-1 IgGs (OR 0.16 [95%CI 0.01-0.36]; $p$=0.009).

# Figure 3



Waist circumference reduction

Waist circumference T0

Weight T0

BMI T0

Hypertension

HDL-c T0

Anti-ApoA-1 Ab T0

Adherence to MD

0.001    0.01    0.1    1    10

OR (95% CI)

## 3. Discussion

The novel and most important finding in the present study is that anti-ApoA-1 IgG levels upon study inclusion were predictors of WC resistance in patients following a MD intervention. Similar resistance to BMI, epicardial ectopic fat, and visceral fat losses during follow-up were observed in anti-apoA-1 IgGs seropositive individuals. This convergent body of systemic observations bears the concept that these autoantibodies could constitute a systemic disruptor of body fat distribution. This hypothesis is supported by recent findings indicating that anti-apoA-1 IgG inhibit the net cholesterol efflux out of the cells and by redirecting the free cholesterol pools toward cellular esterified cholesterol pools due to an increase in ACAT1 activity [8]. This process also involves an anti-apoA-1 IgG-induced activation of cholesterol synthesis and serum LDL-c uptake by inducing the expression of HMG-CoA reductase and LDL-receptor, respectively [8]. Importantly, most of these effects were found to be toll-like receptor (TLR)-2 and -4-dependent [8]. Consistent with the current paradigm considering anti-apoA-1 IgGs as damage-associated molecular pattern (DAMP) eliciting sterile inflammation by activating TLR2/4/CD14 complex, the contribution of these autoantibodies to fat-loss resistance through pathogen related receptors (PRR) activation to sustain chronic low-grade inflammation is likely. Indeed, the contributions of low-grade and chronic inflammation through TLR4 or other PRR activation have been shown to underlie obesity and related metabolic diseases development [13]. Furthermore, once activated by lipid metabolites (e.g. saturated long-chain fatty acids, ceramides, and modified low-density lipoproteins), adipocyte-specific PRRs activation could exert further detrimental effects through oxidative stress, insulin resistance, and/or pro-inflammatory macrophage polarization toward M1 phenotype. Although appealing, the molecular links linking these autoantibodies and fat-loss resistance in

humans needs to be further investigated, as most of the anti-apoA-1 IgG effects reported so far were tested on human macrophages and not on adipocytes [8,9].

Furthermore, it should be considered that many inflammatory pathways may influence – and be influenced by – lifestyle modification programs [14,15]. Among them endocannabinoid system has been demonstrate to be closely linked with adipose tissue metabolism. Whether anti-apoA-1 IgG interact with endocannabinoid system still remains an interesting topic to be investigate in the next future.

From a clinical point of view, these results may open new perspectives regarding patient adherence to diet, which is known to be suboptimal even in the context of supervised lifestyle interventions. In our study, despite the fact that the adherence to MD was quite low, more than half of patients still experienced a reduction of WC, independently of metabolic status at baseline. We assume that if we could have obtained a better compliance to MD, the proportion of patient achieving a WC reduction would have been substantially higher. Currently, the best predictor of adherence to lifestyle interventions is baseline WC (and BMI) at baseline, where overweight individuals are more likely to have low/no adherence to Mediterranean lifestyles. Therefore, any additional tool to facilitate patient motivation and adhesion to lifestyle interventions would fill an unmet clinical need.

This study has several limitations. Firstly, the relatively limited sample size did not allow to reach an adequate study power and to perform multivariate logistic regression analyses. Hence, our results should be considered as preliminary. However, that power issue does not affect the interpretation of significant results. Secondly, due to this size limitation, we cannot exclude that our study population might not be representative of the general population due the monocentric design of the study. Nevertheless, the anti-ApoA-1 IgG seropositivity prevalence and the inverse

associations retrieved with serum LDL-c are in line with prior reports, further confirming the representatively of our cohort. Thirdly, we did not provide any *in vitro* experiment supporting our conclusion about the pathophysiological involvement of those antibodies in obesity and metabolic disorders. Therefore, we cannot exclude that anti-ApoA-1 IgG are only an epiphenomenon and any conclusion or hypothesis requires further validation. However, the lack of correlations observed at baseline would indicate anti-ApoA-1 IgG generation and obesity/MetS as two independent processes, whose interactions surface later during intervention. Furthermore, one year of follow-up is likely a short-time interval to define as stable the reduction of WC. Long-term prospective studies are warranted to confirm these preliminary evidences. Finally, knowing whether the present findings apply to other strategies aiming at weight loss reduction (such as lifestyle interventions and bariatric surgery) requires further investigations.

In conclusion, in patients following MD, high levels of anti-ApoA-1 IgGs are predictive of a poorer weight loss, independently of patients' compliance and systemic inflammation. Due to their substantial discriminant accuracy, those autoantibodies could represent a promising biomarker susceptible to identify a subset of patients particularly resistant to weight-loss reduction while undertaking lifestyle intervention. Although the clinical implications of these findings are still elusive and require further validation, this study extends current knowledge in the field by relating of humoral autoimmunity to body fat distribution and related metabolic disorders.

**Conflict of interest:** The authors report no relationships that could be construed as a conflict of interest.

## Acknowledgments

**References**

1.      Neeland IJ, Poirier P, Despres JP. Cardiovascular and Metabolic Heterogeneity of Obesity: Clinical Challenges and Implications for Management. *Circulation.* 2018;137(13):1391-1406.
2.      Vecchie A, Dallegri F, Carbone F, et al. Obesity phenotypes and their paradoxical association with cardiovascular diseases. *European journal of internal medicine.* 2018;48:6-17.
3.      Ferrara D, Montecucco F, Dallegri F, Carbone F. Impact of different ectopic fat depots on cardiovascular and metabolic diseases. *Journal of cellular physiology.* 2019;234(12):21630-21641.
4.      Carbone F, Nulli Migliola E, Bonaventura A, et al. High serum levels of C-reactive protein (CRP) predict beneficial decrease of visceral fat in obese females after sleeve gastrectomy. *Nutrition, metabolism, and cardiovascular diseases : NMCD.* 2018;28(5):494-500.
5.      Carbone F, Adami G, Liberale L, et al. Serum levels of osteopontin predict diabetes remission after bariatric surgery. *Diabetes & metabolism.* 2019;45(4):356-362.
6.      Carbone F, Elia E, Casula M, et al. Baseline hs-CRP predicts hypertension remission in metabolic syndrome. *European journal of clinical investigation.* 2019;49(8):e13128.
7.      Antiochos P, Marques-Vidal P, Virzi J, et al. Impact of CD14 Polymorphisms on Anti-Apolipoprotein A-1 IgG-Related Coronary Artery Disease Prediction in the General Population. *Arterioscler Thromb Vasc Biol.* 2017;37(12):2342-2349.
8.      Pagano S, Magenta A, D'Agostino M, et al. Anti-ApoA-1 IgGs in Familial Hypercholesterolemia Display Paradoxical Associations with Lipid Profile and Promote Foam Cell Formation. *Journal of clinical medicine.* 2019;8(12).
9.      Vuilleumier N, Pagano S, Montecucco F, et al. Relationship between HDL Cholesterol Efflux Capacity, Calcium Coronary Artery Content, and Antibodies against ApolipoproteinA-1 in Obese and Healthy Subjects. *Journal of clinical medicine.* 2019;8(8).
10.     Carbone F, Lattanzio MS, Minetti S, et al. Circulating CRP Levels Are Associated with Epicardial and Visceral Fat Depots in Women with Metabolic Syndrome Criteria. *International journal of molecular sciences.* 2019;20(23).
11.     Alberti KG, Eckel RH, Grundy SM, et al. Harmonizing the metabolic syndrome: a joint interim statement of the International Diabetes Federation Task Force on Epidemiology and Prevention; National Heart, Lung, and Blood Institute; American Heart Association; World Heart Federation; International Atherosclerosis Society; and International Association for the Study of Obesity. *Circulation.* 2009;120(16):1640-1645.
12.     Sofi F, Macchi C, Abbate R, Gensini GF, Casini A. Mediterranean diet and health status: an updated meta-analysis and a proposal for a literature-based adherence score. *Public health nutrition.* 2014;17(12):2769-2782.
13.     Martinez-Garcia MA, Ojeda-Ojeda M, Rodriguez-Martin E, et al. TLR2 and TLR4 Surface and Gene Expression in White Blood Cells after Fasting and Oral Glucose, Lipid and Protein Challenges: Influence of Obesity and Sex Hormones. *Biomolecules.* 2020;10(1).

14. Di Marzo V, Cote M, Matias I, et al. Changes in plasma endocannabinoid levels in viscerally obese men following a 1 year lifestyle modification programme and waist circumference reduction: associations with changes in metabolic risk factors. *Diabetologia.* 2009;52(2):213-217.

15. Montecucco F, Lenglet S, Quercioli A, et al. Gastric bypass in morbid obese patients is associated with reduction in adipose tissue inflammation via N-oleoylethanolamide (OEA)-mediated pathways. *Thrombosis and haemostasis.* 2015;113(4):838-850.

**Figure Legend**

**Figure 1. Scatter plot illustrating linear regression analysis between serum anti-apolipoprotein A-1 (anti-ApoA-1) IgG and adipose tissue loss during follow-up.** Changes (Δ) in parameters of adiposity during 1-year follow-up in anti-ApoA-1 IgG positive/negative patients: Δ waist circumference (**A**), Δ weight (**B**), Δ body mass index (BMI) (**C**), Δ visceral fat thickness (VFth) (**D**), and Δ epicardial fat thickness (EFth) (**E**).

**Figure 2. Differences in adiposity parameters across patients with anti-apolipoprotein A-1 (anti-ApoA-1) IgG positive/negative during follow-up.** Changes (Δ) in parameters of adiposity during 1-year follow-up in anti-ApoA-1 IgG positive/negative patients: Δ waist circumference (**A**), Δ weight (**B**), Δ body mass index (BMI) (**C**), Δ visceral fat thickness (VFth) (**D**), and Δ epicardial fat thickness (EFth) (**E**).

**Figure 3. Logistic regression showing independent variables associated with waist circumference reduction after 1-year of Mediterranean-like diet.** Data are presented as odds ratio (OR) with 95% of confidence interval (CI). BMI: body mass index; HDL-c: high-density lipoprotein cholesterol; MD: Mediterranean diet.

**Supplementary Figure 1. Metabolic differences in across patients with anti-apolipoprotein A-1 (anti-ApoA-1) IgG positive/negative during follow-up.** Changes (Δ) in metabolic parameters during 1-year follow-up in anti-ApoA-1 IgG positive/negative patients: Δ total cholesterol (total-c) (**A**), Δ low-density lipoprotein (LDL-c) (**B**), Δ high-density lipoprotein cholesterol (HDL-c) (**C**), Δ triglycerides (TAG) (**D**), Δ glycemia (**E**), and Δ visceral adiposity index (VAI) (**F**).
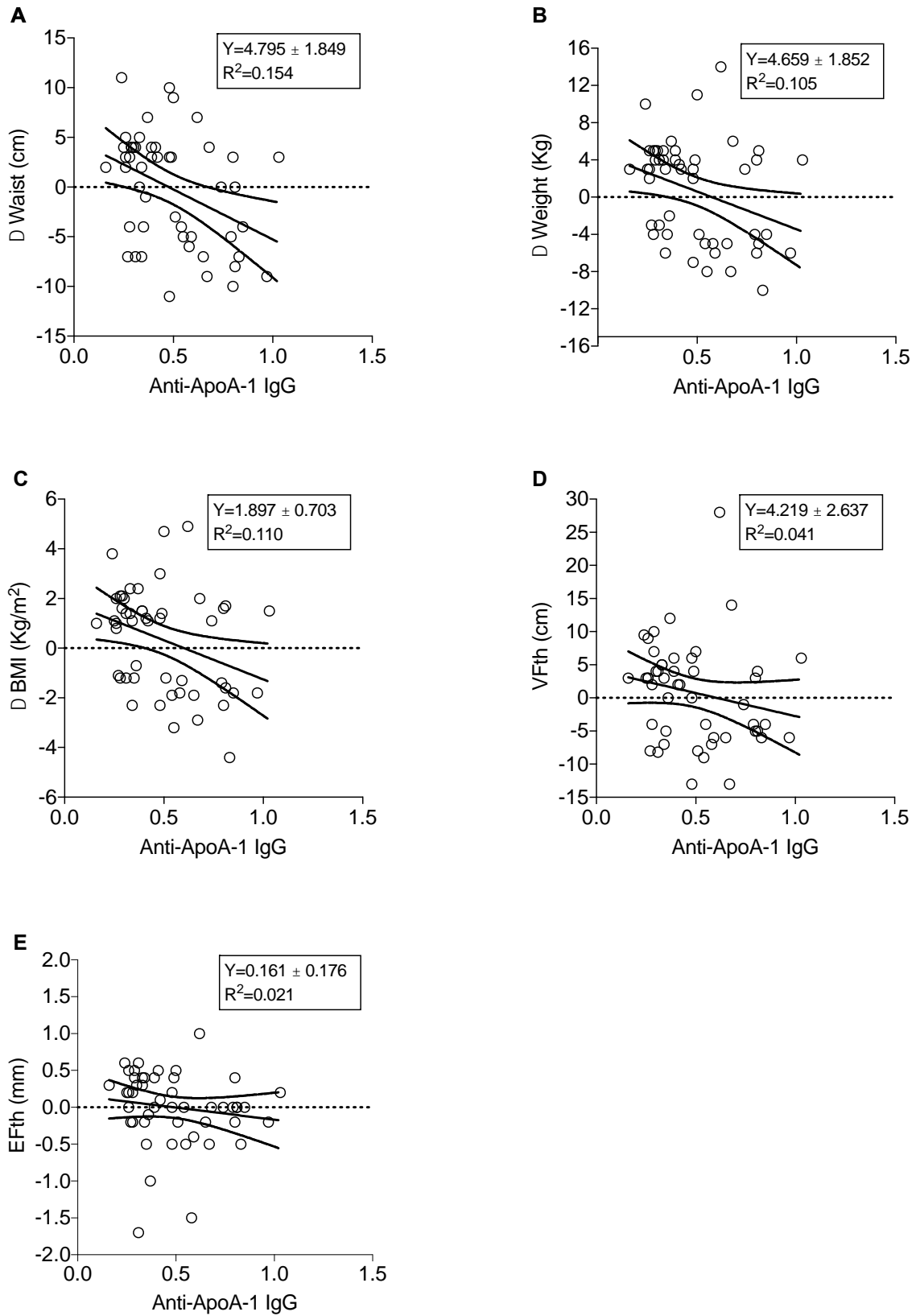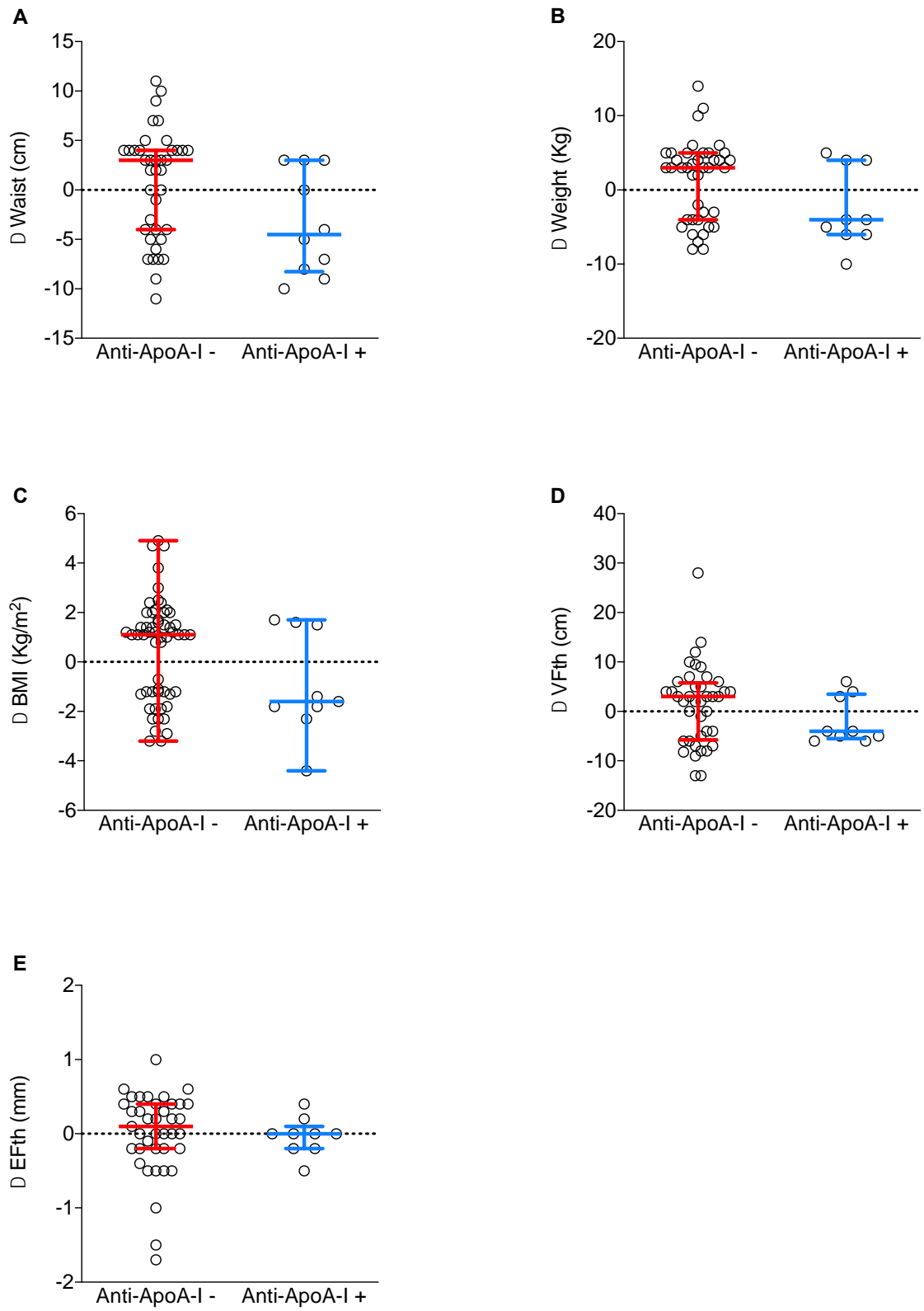
# Figure 1

**A**

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**D**

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VFth (cm) vs Anti-ApoA-1 IgG

**E**

Y=0.161 ± 0.176
$R^2$=0.021

EFth (mm) vs Anti-ApoA-1 IgG

# Figure 2

# Figure 3



Waist circumference reduction