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# Dynamic regulation of circulating endotrophin by changes in fat mass in humans

Rodrigo Fernández-Verdejo [a,b], Dawei Bu [c], Ningyan Zhang [d], Zhiqiang An [d], Eric Ravussin [b,*], Philipp E. Scherer [c,**]

[a] Departamento de Nutrición y Dietética, Escuela de Ciencias de la Salud, Facultad de Medicina, Pontificia Universidad Católica de Chile, Santiago, 8331150, Región Metropolitana, Chile
[b] Pennington Biomedical Research Center, Louisiana State University, Baton Rouge, LA, 70808, USA
[c] Touchstone Diabetes Center, University of Texas Southwestern, Dallas, TX, 75390-8549, USA
[d] Texas Therapeutics Institute, Brown Foundation Institute of Molecular Medicine, University of Texas Health Science Center at Houston, Houston, TX, 77030, USA

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ABSTRACT

*Background and aims:* During long-term weight gain, extracellular matrix remodeling in adipose tissue elevates local and systemic endotrophin, potentially contributing to metabolic dysfunction. Whether short-term increases or reductions in fat mass modulate circulating endotrophin concentrations in humans remains unclear. We aimed to determine the relationship between short-term changes in fat mass (kg and fat percentage points) and circulating endotrophin levels, and to explore whether endotrophin mediates the effects of adiposity on lipid profiles.

*Methods:* Secondary analysis of the pooled individual participant data from two clinical trials. One trial randomized 42 healthy adults (52.4% women, 26–49 years) to one of the following six-month interventions: (a) weight maintenance, (b) 25% caloric restriction, (c) 12.5% caloric restriction and 12.5% increased energy expenditure, or (d) very low-calorie diet. The other trial enrolled 31 healthy adults (19.4% women, 20–40 years) and exposed them to an eight-week 40% overfeeding. Fat mass, circulating endotrophin, and circulating lipid profile were measured before and after these interventions.

*Results:* Considering all participants, the changes in fat mass ranged from −11.0 to +7.0 kg or −9.6 to +5.7 percentage points. Changes in fat mass were positively associated with changes in circulating endotrophin (unstandardized B-coefficient [95% CI] = 0.76 [0.45, 1.07] ng/mL/kg and 0.89 [0.51, 1.28] ng/mL/percentage point, $P < 0.001$). Exploratory mediation analyses showed that endotrophin mediated 15% [4, 51] of the effect of fat mass on total cholesterol.

*Conclusion:* Changes in fat mass, including loss and gain, are associated with corresponding changes in circulating endotrophin, which may partially mediate metabolic effects. Future studies should test whether endotrophin represents a therapeutic target for metabolic health in humans.

## 1. Introduction

Excessive accumulation of fat mass is associated with disruption of organ and tissue function and increased mortality risk [1–3]. Nevertheless, fat mass per se might be insufficient to fully explain the health impairment in humans. A large cohort study reported that, after adjusting for body mass index (BMI) and waist-to-hip ratio, fat mass percentage was not associated with cardiovascular disease or all-cause mortality [4]. Similar findings emerged from two of our clinical trials, in which the extent of decrease in fat mass percentage was not associated with cardiometabolic outcomes after adjusting for changes in body weight [5]. These observations suggest that the adverse effects of fat accumulation depend on the extent to which it disrupts the function of adipose tissue and other tissues. Identifying molecular mediators linking fat mass and cardiometabolic health could guide the development of novel therapeutic targets for obesity treatment.

Endotrophin has been proposed as a potential mediator of the association between excessive fat mass and cardiometabolic health [6].

* Correspondence to: E. Ravussin, Pennington Biomedical Research Center, 6400 Perkins Road, Baton Rouge, LA, 70808, USA.
** Correspondence to: P. E. Scherer, Touchstone Diabetes Center, 5323 Harry Hines Boulevard, Dallas, TX, 75390-8549, USA.
*E-mail addresses:* eric.ravussin@pbrc.edu (E. Ravussin), philipp.scherer@utsouthwestern.edu (P.E. Scherer).

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Endotrophin is a peptide derived from the cleavage of the carboxyl-terminal of collagen type VI alpha-3 chain. Prolonged positive energy balance promotes the accumulation of fat within adipocytes. To support increasingly larger adipocytes, the extracellular matrix of adipose tissue undergoes remodeling with the synthesis of collagen VI microfibrils that produce endotrophin (reviewed in [6]). Hypertrophic adipocytes eventually develop hypoxia, which promotes fibrosis and increases collagen VI and endotrophin levels (reviewed in [7]). In mice, endotrophin has been shown to upregulate pro-fibrotic and pro-inflammatory genes in adipose tissue [8]. In humans, circulating endotrophin has been positively associated with the expression of fibrosis- and inflammation-related genes in adipose tissue [9]. Fibrosis may restrict adipose tissue expansion, redirecting lipids to ectopic adipose storage. Combined with inflammation, this may impair the function of other tissues and drive cardiometabolic disturbances.

Animal and human studies support the influence of endotrophin on cardiometabolic health. Oh et al. [10] exposed mice with adipose tissue-specific overexpression of endotrophin and wild-type controls to a high-fat diet for 23 weeks. Both groups gained similar body weight, but transgenic mice showed higher expression of inflammatory genes in adipose tissue, poorer glucose tolerance, and lower insulin sensitivity. Sun et al. [8] treated high-fat-diet-fed mice with or without an anti-endotrophin antibody for six weeks. Despite similar weight gain, mice treated with anti-endotrophin antibodies exhibited less adipose tissue inflammation and fibrosis, lower circulating and hepatic triglycerides, and greater insulin sensitivity. Furthermore, Yoshiji et al. [11] showed, via Mendelian Randomization, causal and positive associations between BMI and circulating endotrophin, and between circulating endotrophin and coronary artery disease. Observational, longitudinal studies in the same report confirmed those associations [11]. Additional Mendelian Randomization analyses showed that changes in fat mass percentage influenced circulating endotrophin concentrations and coronary disease risk [11]. In the same line, Smith and Klein [9] reported higher circulating endotrophin concentrations in individuals with obesity and impaired insulin sensitivity than in those with similar obesity but high insulin sensitivity. These authors also showed that endotrophin directly impairs insulin sensitivity in primary human myotubes in vitro. Additionally, they reported that individuals with obesity and type 2 diabetes who lost >15% body weight by bariatric surgery or diet showed decreases in circulating endotrophin. Overall, published data suggest that endotrophin mediates some of the effects of fat mass on cardiometabolic health.

Analyses of observational data suggest that changes in fat mass may influence circulating endotrophin concentrations in humans [11]. Consistent with this idea, a clinical trial demonstrated that weight loss reduces circulating endotrophin concentrations in individuals with obesity and type 2 diabetes [9]. Nevertheless, it remains unknown whether short-term interventions that decrease or increase fat mass induce corresponding changes in circulating endotrophin in healthy humans. We hypothesized that changes (decreases and increases) in fat mass are directly associated with changes in circulating endotrophin concentrations in humans. The primary aim of this study was to determine the association between changes in fat mass and changes in systemic endotrophin concentration in humans undergoing caloric restriction or overfeeding. As an exploratory aim, we also tested whether changes in circulating endotrophin concentration mediate part of the effects of the changes in fat mass on lipid profile.

## 2. Methods

### 2.1. Study design

This report is a secondary analysis from the CALERIE 1 (NCT00099151) [12] and EAT (NCT01672632) [13] studies conducted at Pennington Biomedical Research Center (Baton Rouge, LA, USA). For the current analyses, we included participants with body composition data and available blood samples for endotrophin measurement before and after the interventions. In CALERIE 1 (March 2002 to May 2006), 42 individuals were randomized to one of the following interventions for six months: (a) weight maintenance diet; (b) 25% caloric restriction based on baseline energy requirements; (c) 12.5% caloric restriction and 12.5% increase energy expenditure through exercise, based on baseline energy requirements; or (d) very low-calorie diet (890 kcal/day) until 15% weight loss, followed by weight maintenance. In EAT (May 2008 to February 2012), 31 individuals underwent 40% overfeeding above baseline energy requirements for eight weeks. We pooled the individual participant data from these two studies to capture a broad range of experimentally-induced changes in fat mass, including both decreases (CALERIE 1) and increases (EAT). As this is a secondary analysis from one randomized controlled trial (CALERIE 1) and one non-randomized trial (EAT), the analyses are observational in nature. Therefore, the reporting follows the STROBE guidelines (see checklist in Supplementary Table 1) [14].

### 2.2. Participants

Eligibility criteria for CALERIE 1 and EAT have been published [12,13] (NCT00099151 and NCT01672632, respectively). Briefly, the CALERIE 1 study enrolled healthy adults aged 25–50 years with a BMI of 25 to <30 kg/m$^2$; the EAT study enrolled healthy adults aged 18–40 years with a BMI of 22.5 to 32.5 kg/m$^2$. Both studies were approved by the Pennington Institutional Review Board, and all participants provided written informed consent.

### 2.3. Body composition

Absolute and relative fat mass were assessed using dual-energy X-ray absorptiometry (QDR 4500A; Hologic, Bedford, MA). Fasting body weight was measured wearing a weighed gown on a calibrated scale. Fat mass (kg) was calculated as the product of fat mass percentage (%) and body weight (kg) divided by 100. Fat-free mass (kg) was calculated as the difference between body weight (kg) and calculated fat mass (kg).

### 2.4. Circulating lipids and endotrophin

Fasting serum lipids were analyzed using a Beckman-Coulter Synchron CX7 (Brea, CA). Total cholesterol was measured using the cholesterol/esterase/oxidase/peroxidase method. High-density lipoprotein cholesterol (HDL-C) was measured after precipitation of apoB-containing lipoproteins with dextran sulfate (50,000 mol. wt.; Sigma Chemical Co.). Triglycerides were measured using the GPO-Trinder method. Low-density lipoprotein cholesterol (LDL-C) was calculated using the Friedewald equation.

Circulating endotrophin was measured from fasting plasma samples in CALERIE 1, and from serum collected during the low-dose insulin infusion phase of a two-step hyperinsulinemic-euglycemic clamp in EAT (10 mU/min/m$^2$ insulin, glycemia maintained at 90 mg/dL) [13]. Previous data showed that circulating endotrophin concentrations remain stable despite marked insulin fluctuations throughout the day [9]; thus, the clamp was not expected to affect circulating endotrophin concentrations. Endotrophin was quantified as previously described [15]. Briefly, 96-well plates (Corning Costar) were coated with in-house humanized monoclonal anti-endotrophin antibodies, incubated overnight at 4 °C, and blocked with 3% BSA/PBS for 1 h at room temperature. Samples were diluted in PBS, added to anti-endotrophin-coated plates, and incubated for 1 h at room temperature. A high-affinity human-specific anti-endotrophin antibody (ETN-1Rb) was used as a secondary antibody, with an anti-Rabbit Fab2-HRP antibody (Jackson ImmunoResearch) used to detect endotrophin signals. A standard curve was generated using recombinant endotrophin (HIS-tagged) produced in 293-T cells. TMB substrate (Thermo Fisher) was used for immunoreactions, stopped with 2 M sulfuric acid, and read at 450 nm.

### 2.5. Analyses

Analyses were performed in R version 4.5.0 (2025-04-11) using RStudio version 2026.01.1 + 403. Statistical significance was set at $P < 0.05$. Numerical variables are reported as medians with 25th and 75th percentiles, and categorical variables as frequencies.

Wilcoxon tests (*wilcox.test* function) were used to determine whether within-group changes (post – baseline) in numerical variables differed from zero and to compare baseline circulating endotrophin concentrations between CALERIE 1 and EAT participants. Linear models (*lm* function) were used to test the associations between changes in fat mass (kg or fat percentage points) and changes in endotrophin, adjusting for baseline values. Shapiro-Wilk tests (*shapiro.wilk* function) and visual inspection of histograms were used to assess the normal distribution of model residuals. Studentized Breusch-Pagan tests (*bptest* function, lmtest package [16]) and visual inspection of residual-versus-predicted plots were used to test against heteroskedasticity of model residuals. Sensitivity analyses included fitting the models using ranked values to approach a normal distribution and homoscedasticity of model residuals, adding a study-by-changes in fat mass interaction term, or adding sex and age as additional covariates. Associations were visualized by plotting partial residuals of the changes in endotrophin against the changes in fat mass, while fixing covariates at their median values (baseline fat mass: 21.6 kg or 25.8%; baseline endotrophin: 15.3 ng/mL), as previously described [17]. Linear models were also used to test associations between changes in fat mass and changes in lipid profile (total cholesterol, LDL-C, HDL-C, and triglycerides), adjusting for baseline values. Associations between variables are presented as unstandardized B-coefficients with 95% confidence intervals (95% CI).

Exploratory mediation analyses were conducted using the *mediate* function from the *mediation* package [18]. We estimated average causal mediation effects and the proportion mediated by changes in circulating endotrophin concentration in the relationship between changes in fat mass and changes in lipid profile. The outcomes were changes in lipid profile variables; the treatment was changes in fat mass (kg and fat percentage points); the mediator was changes in circulating endotrophin concentration; and the covariates were the baseline values for all variables. Sex and age were included as additional covariates in sensitivity analyses. Mediation effects and variance were estimated using bias-corrected and accelerated intervals based on 5000 non-parametric bootstrap simulations. Reporting of mediation analyses followed the AGReMA guidelines (Supplementary Table 2) [19].

## 3. Results

### 3.1. Baseline participant characteristics and the impact of the interventions

Table 1 summarizes baseline characteristics by intervention. Overall, the sample included 45 men and 28 women aged 20–49 years, with BMI from 22.5 to 31.4 kg/m$^2$. At baseline, fat mass ranged from 7.9 to 36.2 kg, fat mass percentage from 10.4% to 42.6%, and circulating endotrophin from 2.4 to 35.2 ng/mL. Fat mass (kg or percentage points) was not associated with circulating endotrophin concentration at baseline (Fig. 1). Finally, circulating endotrophin concentrations at baseline were higher in participants from the EAT study than in those from the CALERIE 1 study (median [25th percentile, 75th percentile]; 16.8 [12.3, 22.0] versus 13.6 [9.44, 19.5] ng/mL, respectively; $P = 0.042$).

Table 2 shows the intervention effects on body weight and lipid profile. Body weight decreased with caloric restriction, with the combination of caloric restriction and exercise, and with the very low-calorie diet, while increasing with overfeeding. Total cholesterol increased in the weight maintenance and overfeeding groups but decreased with caloric restriction and with the combination of caloric restriction and exercise. HDL-C increased, and triglycerides decreased with caloric restriction and with the combination of caloric restriction and exercise. LDL-C decreased with the combination of caloric restriction and exercise and with the very low-calorie diet, but increased with overfeeding.

### 3.2. Intervention effects on fat mass and endotrophin

As expected, fat mass (kg and percentage points) remained unchanged in the weight maintenance group, decreased with caloric restriction, with the combination of caloric restriction and exercise, and with the very low-calorie diet, and increased with overfeeding (Fig. 2A–B). Circulating endotrophin increased in the weight maintenance and overfeeding groups, decreased with caloric restriction and with the combination of caloric restriction and exercise, and remained unchanged with the very low-calorie diet (Fig. 2C). Considering the participants from all groups together, changes in fat mass were positively associated with changes in circulating endotrophin (Fig. 2D–E). For every kilogram or percentage point change in fat mass, circulating

**Table 1**
Baseline characteristics of the participants by intervention.

| Variable | Weight maintenance (n = 11) | | | Caloric restriction (n = 11) | | | Caloric restriction and exercise (n = 11) | | | Very low-calorie diet (n = 9) | | | Overfeeding (n = 31) | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Mdn[a] | p25 | p75 | Mdn | p25 | p75 | Mdn | p25 | p75 | Mdn | p25 | p75 | Mdn | p25 | p75 |
| Sex | | | | | | | | | | | | | | | |
| Men, n | 5 | – | – | 6 | – | – | 5 | – | – | 4 | – | – | 25 | – | – |
| Women, n | 6 | – | – | 5 | – | – | 6 | – | – | 5 | – | – | 6 | – | – |
| Age, y | 40 | 32 | 44 | 40 | 35 | 43 | 36 | 32 | 40 | 40 | 35 | 44 | 25 | 22 | 29 |
| Race | | | | | | | | | | | | | | | |
| White, n | 7 | – | – | 7 | – | – | 5 | – | – | 7 | – | – | 18 | – | – |
| AA, n | 4 | – | – | 3 | – | – | 4 | – | – | 2 | – | – | 13 | – | – |
| Other, n | 0 | – | – | 1 | – | – | 2 | – | – | 0 | – | – | 0 | – | – |
| Height, m | 1.72 | 1.66 | 1.79 | 1.73 | 1.68 | 1.75 | 1.73 | 1.66 | 1.77 | 1.71 | 1.63 | 1.73 | 1.76 | 1.71 | 1.81 |
| Weight, kg | 79.9 | 76.4 | 85.7 | 80.5 | 79.0 | 87.8 | 81.0 | 77.4 | 84.0 | 74.6 | 72.3 | 79.9 | 80.2 | 70.8 | 86.6 |
| BMI, kg/m$^2$ | 26.9 | 26.2 | 29.0 | 27.5 | 26.7 | 29.4 | 26.8 | 26.2 | 28.6 | 27.3 | 26.7 | 28.0 | 24.6 | 23.7 | 27.2 |
| Fat mass, % | 32.1 | 26.0 | 35.1 | 29.1 | 25.2 | 38.0 | 32.1 | 26.1 | 36.9 | 32.5 | 26.3 | 38.1 | 20.6 | 15.7 | 24.8 |
| Fat mass, kg | 25.4 | 21.9 | 27.3 | 26.1 | 20.5 | 29.3 | 22.4 | 21.7 | 29.6 | 23.8 | 21.3 | 28.1 | 18.2 | 11.9 | 20.7 |
| FFM, kg | 54.0 | 48.0 | 63.7 | 60.1 | 48.8 | 66.5 | 52.7 | 47.2 | 61.0 | 48.8 | 44.8 | 63.7 | 63.7 | 56.2 | 68.5 |
| Endotrophin, ng/mL | 14.2 | 11.1 | 16.0 | 12.4 | 9.6 | 21.6 | 12.4 | 9.8 | 18.9 | 14.6 | 7.8 | 21.2 | 16.8 | 12.3 | 22.0 |
| Cholesterol, mg/dL | 172.3 | 161.5 | 180.8 | 179.0 | 165.5 | 198.0 | 172.0 | 148.0 | 197.5 | 181.0 | 175.3 | 201.0 | 168.0 | 155.0 | 183.5 |
| HDL-C, mg/dL | 36.3 | 29.7 | 39.8 | 44.2 | 36.5 | 46.6 | 45.9 | 35.9 | 50.4 | 38.0 | 33.8 | 51.7 | 56.6 | 48.4 | 64.7 |
| LDL-C, mg/dL | 108.0 | 100.0 | 124.3 | 109.8 | 93.2 | 123.3 | 104.6 | 88.5 | 119.4 | 108.0 | 106.0 | 134.1 | 95.8 | 85.7 | 109.9 |
| Triglycerides, mg/dL | 115.0 | 90.5 | 170.4 | 124.0 | 76.7 | 188.2 | 84.0 | 58.5 | 127.3 | 119.0 | 76.0 | 130.0 | 74.0 | 55.0 | 96.0 |

[a] Data for sex and race are frequencies. AA, African American; BMI, body mass index; FFM, fat-free mass; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; Mdn, median; p25, 25th percentile; p75, 75th percentile.

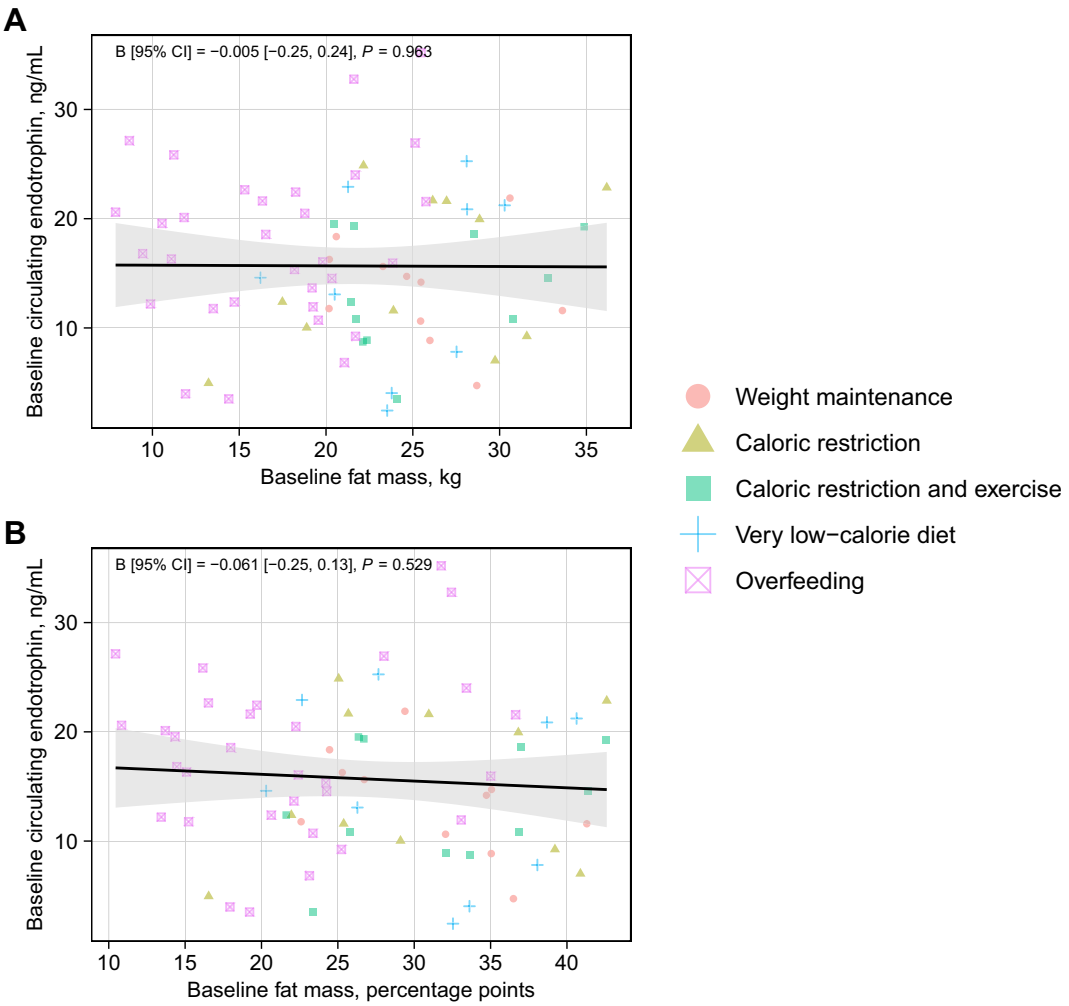


**Fig. 1.** Association between fat mass and circulating endotrophin at baseline. (A) Fat mass in kilograms. (B) Fat mass in percentage points. B, unstandardized B-coefficient; 95% CI, 95% confidence intervals. $n = 11$ for weight maintenance; n = 11 for caloric restriction; $n = 11$ for caloric restriction and exercise; $n = 9$ for very low-calorie diet; and $n = 31$ for overfeeding.

endotrophin changed by a mean [95% CI] of 0.76 [0.45, 1.07] or 0.89 [0.51, 1.28] ng/mL, respectively.

Model residuals showed deviations from a normal distribution according to the visual inspection of histograms and Shapiro-Wilk tests (model with fat mass in kg: W = 0.95, $P = 0.012$; model with fat mass in percentage points: W = 0.95, $P = 0.017$). Model residuals also showed some heteroskedasticity, as indicated by visual inspection of residual-versus-predicted plots and studentized Breusch-Pagan tests (model with fat mass in kg: BP = 10.75, $P = 0.013$; model with fat mass in percentage points: BP = 11.14, $P = 0.010$). Notably, after ranking the numerical variables to approach a normal distribution and homoscedasticity of model residuals, the associations between fat mass and circulating endotrophin remained significant ($P < 0.001$). When the study-by-changes in fat mass interaction term was included in the models, neither the interaction term nor the study term (CALERIE 1, EAT) was statistically significant ($P > 0.11$). In these models, the association between changes in fat mass and changes in circulating endotrophin remained statistically significant ($P < 0.05$). Finally, including sex and age as covariates did not alter associations, and these additional covariates were not statistically significant in the models ($P > 0.40$).

### 3.3. Mediation of endotrophin on the effects of fat mass on lipid profile

One participant in the caloric restriction group lacked LDL-C data, and one in the overfeeding group lacked the entire lipid profile (Table 2). These participants were excluded from analyses that included those variables. Changes in fat mass (kg) were positively associated with changes in total cholesterol (B = 2.90 mg/dL/kg, $P < 0.001$, $n = 72$), LDL-C (B = 2.15 mg/dL/kg, $P < 0.001$, $n = 71$), and triglycerides (B = 3.29 mg/dL/kg, $P = 0.008$, n = 72). Similar associations were observed for the changes in fat mass expressed as percentage points (total cholesterol: B = 3.35 mg/dL/percentage point, $P < 0.001$, n = 72; LDL-C: B = 2.54 mg/dL/percentage point, $P < 0.001$, n = 71; triglycerides: B = 3.41 mg/dL/percentage point, $P = 0.026$, n = 72). No associations between fat mass and HDL-C were observed (B = −0.03 mg/dL/kg, $P = 0.841$, n = 72; B = −0.07 mg/dL/percentage point, $P = 0.718$, n = 72).

The exploratory mediation analyses were thus conducted for total cholesterol, LDL-C, and triglycerides (Fig. 3). We considered the following assumptions: [a] the intervention-induced changes in fat mass occurred before the changes in circulating endotrophin and lipid profile [6,7,11]; [b] the changes in fat mass caused the changes in circulating endotrophin and lipid profile [11]; and [c] there were no unmeasured confounding factors between the changes in circulating endotrophin and the changes in lipid profile. Thus, endotrophin mediated [95% CI] 15% [4, 53] of the effect of fat mass percentage on total cholesterol (Fig. 3D). A similar pattern was observed for the changes in fat mass in kilograms, but it did not reach significance (12% [1, 42], $P = 0.075$; Fig. 3A). No other mediation effects were detected. Results were similar in sensitivity analyses that included sex and age as additional covariates (not shown).

**Table 2**
Changes (post – baseline) in body weight and lipid profile with the interventions.

| Variable | Weight maintenance (n = 11) | | | | Caloric restriction (n = 11) | | | | Caloric restriction and exercise (n = 11) | | | | Very low-calorie diet (n = 9) | | | | Overfeeding (n = 31) | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Mdn | p25 | p75 | *P*-value | Mdn | p25 | p75 | *P*-value | Mdn | p25 | p75 | *P*-value | Mdn | p25 | p75 | *P*-value | Mdn | p25 | p75 | *P*-value |
| Weight, kg | −1.0 | −2.1 | 2.6 | 0.966 | −9.3 | −10.5 | −6.8 | 0.001 | −8.3 | −10.3 | −6.3 | 0.001 | −11.2 | −12.3 | −10.4 | 0.004 | 7.4 | 5.6 | 8.7 | <0.001 |
| Cholesterol, mg/dL[a] | 7.7 | 0.7 | 15.2 | 0.019 | −10.5 | −12.8 | −1.2 | 0.019 | −9.0 | −28.5 | −5.5 | 0.005 | −10.0 | −26.5 | −9.3 | 0.058 | 16.5 | 8.3 | 33.5 | <0.001 |
| HDL-C, mg/dL[a] | 2.1 | −0.5 | 4.7 | 0.067 | 4.6 | 3.3 | 6.3 | 0.002 | 4.0 | 3.1 | 5.3 | 0.010 | 4.6 | −2.5 | 10.9 | 0.129 | 1.5 | −2.1 | 6.1 | 0.102 |
| LDL-C, mg/dL[a,b] | 2.9 | −3.8 | 7.8 | 0.610 | −7.4 | −12.0 | −2.7 | 0.160 | −8.6 | −26.1 | −5.6 | 0.002 | −20.9 | −22.5 | −6.4 | 0.027 | 15.1 | 5.7 | 25.3 | <0.001 |
| Triglycerides, mg/dL[a] | 20.0 | −1.0 | 55.5 | 0.100 | −16.5 | −44.4 | −8.9 | 0.024 | −18.7 | −32.1 | −3.3 | 0.014 | −20.0 | −31.7 | 10.3 | 0.164 | −5.0 | −29.5 | 15.0 | 0.593 |

HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; Mdn, median; p25, 25th percentile; p75, 75th percentile.
[a] $n = 30$ in overfeeding.
[b] $n = 10$ in caloric restriction.

## 4. Discussion

We investigated whether short-term increases (overfeeding) or decreases (caloric restriction with or without exercise) in fat mass affect endotrophin circulating concentrations in humans. Our main finding is that changes in fat mass (kg or percentage points) were positively associated with changes in endotrophin, supporting our original hypothesis. Using mediation analyses, we explored whether endotrophin mediates the effects of the changes in fat mass on lipid profile. We found that endotrophin accounted for 15% of the effect of fat mass percentage on total cholesterol, without impact on LDL-C or triglycerides. Our results suggest that perturbations in energy balance, leading to changes in fat mass, promote the production and release of endotrophin, which then potentially mediates changes in lipid profile.

Previous Mendelian Randomization analyses in humans suggested that changes in fat mass should alter circulating endotrophin [11]. The result was partially confirmed by a study reporting decreases in circulating endotrophin after bariatric surgery or diet [9]. Whether the effects were linear and included both fat loss and gain remained unknown. Here, we analyzed data from lifestyle interventions for weight loss, weight maintenance, and weight gain. Fat mass changes ranged from an 11.0 kg decrease to a 7.0 kg increase, corresponding to decreases of 9.6 percentage points to increases of 5.7 percentage points. The weight maintenance group, without changes in fat mass, provided data across the full range of fat mass changes, enabling linear association estimates. We demonstrated, for the first time, that changes in fat mass, spanning decreases and increases, are positively associated with changes in circulating endotrophin in humans. Although we cannot establish a causal relationship between changes in fat mass and changes in circulating endotrophin, the perturbations in energy balance induced by the interventions represent an established method to manipulate fat mass. Previous reports have shown that weight loss downregulates, whereas weight gain upregulates genes related to extracellular matrix remodeling in human adipose tissue [20,21]. Together, evidence suggests that changes in endotrophin are integral to the extracellular matrix remodeling required to sustain changes in fat depots [6,7].

Factors beyond changes in fat mass also seem to influence circulating endotrophin. Although the very low-calorie diet group demonstrated the largest median decrease in fat mass, endotrophin did not change. This group, after achieving the expected 15% weight loss, transitioned to a weight-maintenance diet for ~3 months before post-intervention measurements [12]. In contrast, the caloric restriction and the caloric restriction and exercise groups were measured while maintaining their interventions. The overfeeding group switched to a weight-maintenance diet for only three days before measurements [13]. Thus, post-intervention measures reflected ongoing changes in fat mass across all groups, except the very low-calorie diet group. This observation suggests that changes in circulating endotrophin reflect active extracellular matrix remodeling in adipose tissue. When fat mass stabilizes, endotrophin may return to baseline concentrations, at least in circulation. This idea aligns with the lack of association we observed between fat mass (kg or percentage points) and circulating endotrophin at baseline, when individuals were weight-stable. In agreement, Smith and Klein [9] found similar circulating endotrophin concentrations in insulin-sensitive individuals with lean (28% fat mass) or obesity (51% fat mass) phenotypes. To what extent circulating endotrophin reflects adipose tissue endotrophin in the context of weight stability remains to be determined.

Endotrophin has been proposed as a driver of metabolic disturbances [6]. Mouse studies using endotrophin overexpression in adipose tissue or anti-endotrophin antibodies during weight gain support this idea [8,10], as do human studies using Mendelian Randomization or weight loss interventions [9,11]. Here, we explored the idea using mediation analyses focused on the lipid profile, because circulating endotrophin has been associated with coronary heart disease [11]. In our data, changes in fat mass were related to changes in total cholesterol, LDL-C, and triglycerides. Exploratory mediation analyses revealed that

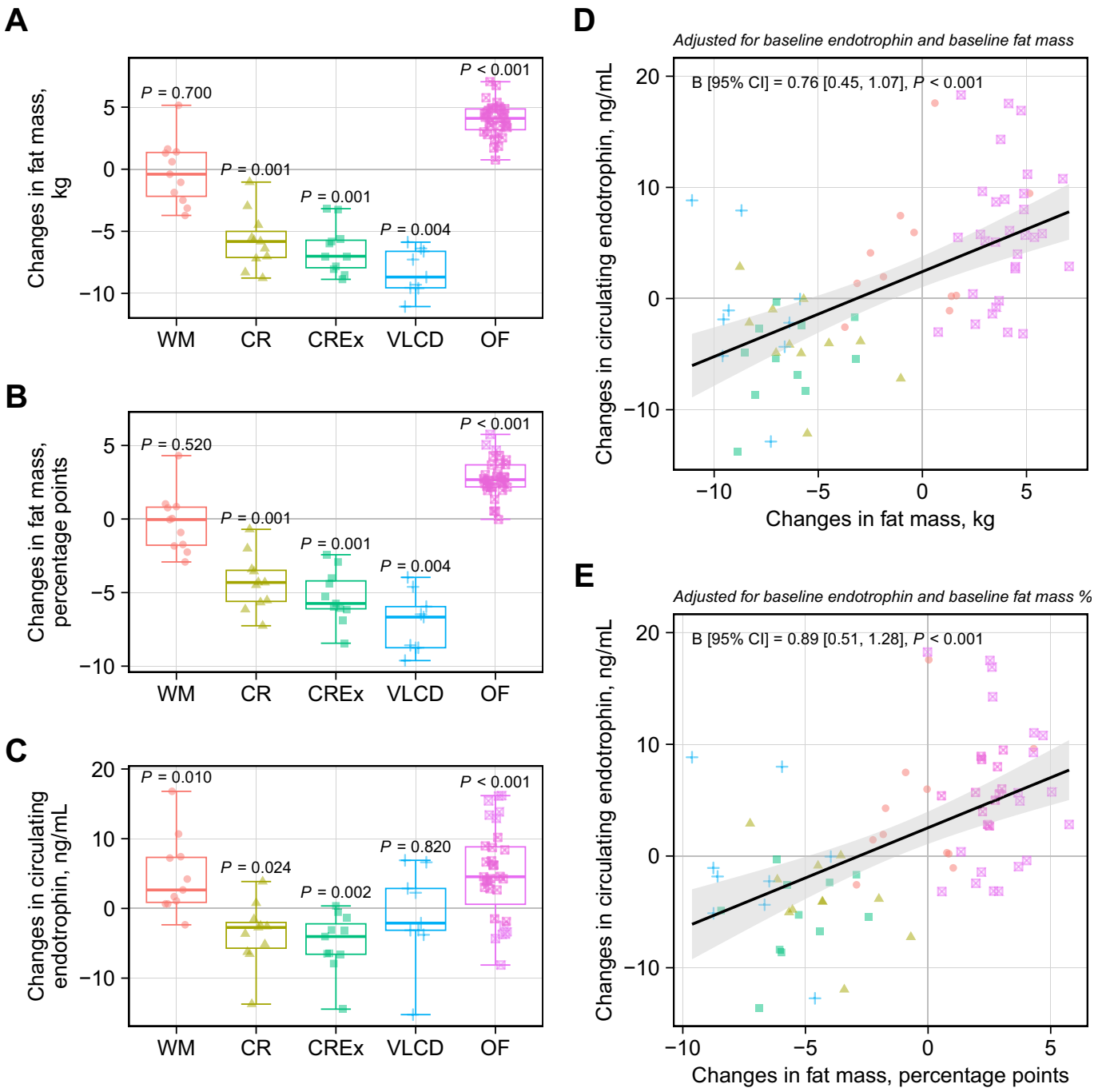


**Fig. 2.** Effects of interventions on fat mass and circulating endotrophin. Changes in (A) fat mass in kilograms, (B) fat mass as percentage points, and (C) circulating endotrophin in individuals exposed to a weight maintenance diet [WM, n = 11], caloric restriction [CR, n = 11], caloric restriction and exercise [CREx, n = 11], very low-calorie diet [VLCD, n = 9], or overfeeding [OF, n = 31] interventions. Association of the changes in fat mass (D) in kilograms and (E) as percentage points with changes in circulating endotrophin [*n* = 73]. B, unstandardized B-coefficient; 95% CI, 95% confidence intervals.

endotrophin potentially mediated 15% of the effect of fat mass on total cholesterol. Note that the confidence intervals for this effect, however, were rather wide, from 4 to 53%. Thus, circulating endotrophin likely reflects one of several biological pathways that mediate the effects of fat mass on cardiovascular health. Greater increases in circulating endotrophin during weight gain may exacerbate adipose tissue fibrosis, limiting adipose tissue expansion, and promoting ectopic lipid accumulation. In agreement, endotrophin overexpression upregulates profibrotic genes in adipose tissue, whereas anti-endotrophin antibodies attenuate fibrosis and hepatic triglyceride accumulation during weight gain in mice [8]. In humans, weight gain upregulates fibrosis-related genes in adipose tissue and increases intrahepatic lipids [13,21]. In contrast, weight loss upregulates extracellular matrix remodeling genes and downregulates cholesterol flux genes in adipose tissue, coinciding with reductions in intrahepatic triglyceride [20].

Our study has limitations. First, as mentioned before, we cannot establish causality between changes in fat mass and changes in endotrophin. Second, the causal relationship between changes in endotrophin and changes in total cholesterol cannot be confirmed. Neither can reverse causality, in which total cholesterol influenced endotrophin, be discarded. Although mediation analyses provide estimated effects, their 95% confidence intervals were relatively wide due to the modest

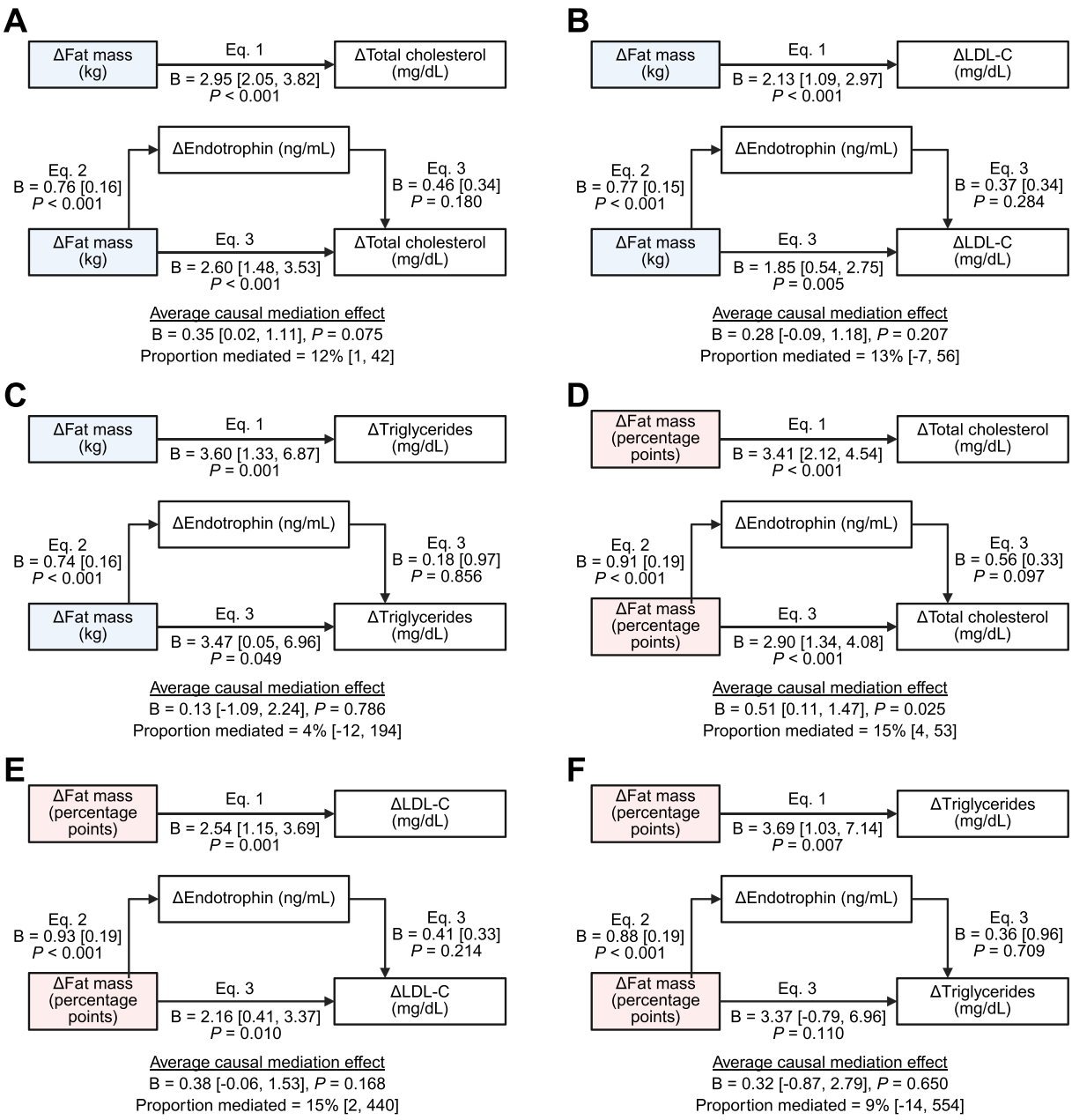


**Fig. 3.** Mediation analyses of circulating endotrophin in the relationship between changes in fat mass and lipid profile. Changes in fat mass (kg: A–C; percentage points: D–F) were considered the treatment variable. All models included baseline values of fat mass (kg or percentage points), circulating endotrophin, and lipid profile component (total cholesterol, low-density lipoprotein cholesterol [LDL-C], or triglycerides) as covariates. Analyses included $n = 72$ for total cholesterol and triglycerides, and $n = 71$ for LDL-C. Uncertainty estimates are standard errors or 95% confidence intervals. B, unstandardized beta coefficient. Created in BioRender. Fernandez verdejo, R. E. (2026) https://BioRender.com/w15fry0.

sample size. Moreover, the mediation analyses had underlying assumptions that could not be verified in our data, so those findings should be considered exploratory and interpreted cautiously. Third, endotrophin influences insulin sensitivity [8–10], but we lacked data on insulin sensitivity and therefore focused on the lipid profile instead. Finally, we combined individual participant data from two studies that included different interventions and sexes, factors that may have influenced the observed results. Studies with larger sample sizes are required to ascertain the effect of each intervention and sex. Sensitivity analyses including the study-by-changes in fat mass interaction or adjusting for sex did not affect our findings.

In conclusion, changes in circulating endotrophin concentration reflect ongoing changes in fat mass and potentially mediate part of the effect of fat on cardiovascular health markers in humans. Targeting endotrophin may help mitigate some of the adverse effects of the progressive weight gain that occurs during adulthood in humans.

## CRediT authorship contribution statement

**Rodrigo Fernández-Verdejo:** Writing – original draft, Visualization, Formal analysis, Data curation. **Dawei Bu:** Writing – review & editing, Methodology, Investigation. **Ningyan Zhang:** Writing – review & editing, Methodology, Funding acquisition. **Zhiqiang An:** Writing – review & editing, Methodology, Funding acquisition. **Eric Ravussin:** Writing – review & editing, Resources, Project administration, Funding acquisition, Conceptualization. **Philipp E. Scherer:** Writing – review & editing, Resources, Project administration, Funding acquisition, Conceptualization.

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## Declaration of competing interest

N.Z., Z.A., and P.S. were inventors of patent applications filed by the University of Texas System covering anti-endotrophin antibodies and their uses. Z.A. and P.S. are scientific cofounders of PriveBio.

## Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.metabol.2026.156679.

## Data availability

The data used in this study are publicly available under the DOI:10.17632/336vjmmtyw.1.

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