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Effect of weight maintenance or gain in a 10 years period over telomere length, sirtuin 1 and 6 expression and carotid intima media thickness

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Abstract

Background: Lack of weight gain throughout adult life could mimic the beneficial effects of energy restriction in humans. The present study aimed to assess the effects of weight stability or gain, over a period of 10 years, on telomere length, sirtuin 1 and 6 expression, and carotid intima media thickness.
Methods: We studied 148 healthy adults (age range 20–59 years; 101 females) who had an objective record of their weight 10 years before. They were classified as weight losers, weight maintainers, weight gainers and extreme weight gainers. A fasting blood sample was obtained for routine laboratory and isolation of peripheral blood mononuclear cells, to extract DNA and RNA, and to measure telomere length and sirtuin 1 and 6 expression, respectively. Carotid intima media thickness was measured by ultrasound. Body composition was measured by Dual-energy X-ray absorptiometry.
Results: In the 10-year period, 24 participants lost weight (17 females), 65 maintained weight (41 females), 25 gained weight (15 females) and 34 were extreme weight gainers (28 females). Female weight gainers had a higher body mass index, waist circumference, total body fat and homeostatic model assessment insulin resistance. Male weight gainers had a higher hip circumference and total body fat. No differences in telomere length, sirtuin 1 expression and carotid intima media thickness were observed between weight gainers and maintainers.
Conclusions: No effect of weight maintenance or gain was observed on metabolic and vascular markers of ageing.

Introduction

Energy restriction (ER) is the most commonly used experimental manipulation used to retard ageing and its consequences in experimental animals (Weindruch & Sohal, 1997). The effects of ER on human ageing are much more complicated to demonstrate. In subjects who were accidentally exposed to energy restriction in the Biosfere 2 experiments, a reduction in serum lipid levels, blood pressure and insulin resistance was observed (Walford *et al.*, 2002). The same effects were reported among members of the Calorie Restriction Society who were voluntarily cutting their energy intake and aiming to increase their longevity (Fontana *et al.*, 2004). The CALERIE trial also observed similar changes after a controlled energy restriction intervention lasting 6 months (Heilbronn *et al.*, 2006). Other attempts have tried to mimic the effects of energy restriction with pharmacological interventions, such as the use of metformin, which reduces weight and insulin resistance. This medication can reduce the incidence of cancer in diabetics and modify gene expression in the same way as energy restriction does (Zhang *et al.*, 2013).

Looking for another approach, we have attempted to develop a retrospective model of energy restriction in humans, comparing different metabolic and functional

parameters between individuals who have maintained a stable weight during a long lapse (i.e. 10 years) compared to subjects who have gained weight. We assume that weight maintainers have sustained or kept a more strict energy balance than gainers. We reported previously that weight maintainers had a lower muscle immunohistochemical expression in muscle of 4-hydroxy nonenal, a marker of oxidative injury, carboxymethyl lysine, an advanced glycation end product, and tumour necrosis factor. However, we did not observe any difference in serum markers between weight maintainers and gainers (De la Maza *et al.*, 2008).

Telomere length in peripheral blood mononuclear cells (PBMC) decreases progressively with age and therefore has been used as a marker of ageing (Aubert & Lansdorp, 2008), whereas sirtuin 1 activity should increase with energy restriction. There is evidence that many of the beneficial effects of this last intervention are mediated by sirtuins (Rahman *et al.*, 2009). Energy restriction in humans increases the expression of SIRT1 in peripheral mononuclear cells (Kitada *et al.*, 2013). There is no evidence of the effect of this intervention on SIRT6, although the latter enzyme plays an important role in the maintenance of telomere length and endothelial function (Cardus *et al.*, 2013).

The present study aimed to investigate differences in ageing markers between a larger group of subjects who had maintained or gained weight in the last 10 years. As ageing markers, we chose telomere length measured in PBMCs and carotid intima media thickness (CIMT) measured by ultrasound. Sirtun 1 and 6 expression in PBMC were measured as targets of energy restriction and could have an important role with respect to avoiding the consequences of ageing.

Materials and methods

Healthy adults of both genders, aged 20–59 years, were invited to participate in the present study. Participants were selected from databases held at the Institute of Nutrition and Food Technology of previous studies or from a database of a preventive medical programme directed at personnel working in airlines (pilots, crew or mechanics). The main inclusion criterion was to have an objective and quantitative record of their weight 10 years ago. This record could comprise an annotation in a clinical record, data from preventive medical examinations, the database of a dual-energy X-ray densitometer used for body composition analysis or information from other healthcare professionals. We also requested, as a back-up to corroborate the information, a full body photograph from 10 years ago, preferably taken in light clothing. We excluded participants who performed intense physical activity, defined as more than 4 h of exercise per week; those who were taking medications that could modify body composition such as adrenal steroids; those who were heavy smokers, heavy alcohol or illicit drug consumers; and those with a body mass index under 18 kg m^{-2}. We also excluded individuals who had, at the moment of the initial assessment or at the moment of recruitment, a chronic life-threatening disease such as cancer or autoimmune disease, as well as endocrine diseases such as hypothyroidism, hyperthyroidism or diabetes. We also excluded participants who reported wide fluctuations in weight during the previous 10 years. The study was approved by INTA's ethics committee.

After providing their written informed consent, participants were subjected to:

- A full clinical assessment, including a complete record of medication use and previous diseases, and a physical examination, including weight, height and blood pressure measurement, in accordance with the recommendations of the National Heart Lung and Blood Institute (Seventh Report of the Joint National Committee on Prevention, Detection, Evaluation, & Treatment of High Blood Pressure, 2013).
- A dietary record using a food frequency questionnaire. Information on intake was processed using bespoke software developed by the authors.
- A record of current physical activity using a validated Spanish version of the short format of the International Physical Activity Questionnaire (IPAQ) (Kim *et al.*, 2013).
- Withdrawal of a fasting blood sample, to measure routine blood chemistry and to obtain PBMC to extract genomic DNA and RNA.
- Body composition analysis using iDEXA equipment (General Electric, Fairfirld, CT, USA). Data on total body fat and fat free mass were obtained.
- Measurement of CIMT using a LOGICQe ultrasound device (General Electric) equipped with a border recognition software. The recommendations of the American Society of Echocardiography were followed (Stein *et al.*, 2009). Briefly, measurements were carried out with the participant in the supine position and a segment of the common carotid artery near the bifurcation was chosen. Using the automated border recognition software, approximately 100 measurements per segment were carried out.

Laboratory analyses and calculations:

- Blood glucose, creatinine, total and high-density lipoprotein cholesterol, triacylglycerol, thyroid-stimulating hormone, thyroxine, triiodothyronine and insulin were measured in a certified clinical laboratory (Vida Integra, Santiago, Chile). Homeostatic model assessment (HOMA) index values were calculated as:

HOMA-IR = (Fasting insulin(μU/mL)*Fasting blood glucose (nM))/22.5 (Matthews *et al.*, 1985)

- Telomere length was measured in genomic DNA extracted from PBMC using the real-time polymerase chain reaction (PCR), as described by Cawthon (2002). The results are expressed as the ratio (T/S ratio) between telomeres and a housekeeping gene. The primer used for telomeres was: 5′-CGG TTT GTT TGG GTT TGG GTT TGG GTT TGG GTT TGG GTT-3′. For the housekeeping gene, the primer was: 5′-CCC ATT CTA TCA TCA ACG GGT ACA A-3′. The intra- and interassay coefficients of variation of the method were 6% and 13%, respectively (similar to the original description of the technique; Cawthon, 2002)
- Sirtuin 1 and 6 expression were measured by real-time PCR in genomic RNA extracted from PBMC. RNA extraction was carried out using the kit RNeasy from Qiagen (Valencia, CA, USA). The primers were: SIRT 1, forward: CGG AAA CAA TAC CTC CAC C, reverse: CAC CCC AGC TCC AGT TAG; SIRT6, forward: GTG GAC GGG CTC CAT GTG, reverse: GTT CCT GCA GGC TCG C; housekeeping gene, forward: CAG CAA GTG GGA AGG TGT AAT CC, reverse CCC ATT CTA TCA TCA ACG GGT ACA A. Samples were read using Light Cycler quantitative PCR equipment from Roche (Mannheim, Germany).
- The level of physical activity was calculated according to the guidelines provided with the short and long forms of the IPAQ questionnaire (https://sites.google.com/site/theipaq/scoring-protocol). The results are expressed as metabolic equivalents min^{-1} $week^{-1}$.
- CIMT was expressed in millimetres. A mean of 100 calculations were used to calculate the mean and maximal thickness value at each side.

Statistical analysis

Data were analysed using STATA, version 12 (StataCorp., College Station, TX, USA). Participants were divided into four groups: weight losers defined as those who lost weight; weight maintainers defined as those who maintained or gained < 10% of their initial weight; weight gainers defined as those that gained > 10% and <16% of their initial weight; and extreme weight gainers as those that gained more than 16% of their initial weight (the value of 16% corresponds to the median percentage weight gain). Because most values had a non-normal distribution, the results are expressed as the median (range). For purposes of analysis, participants were divided by gender and weight change status. Therefore, four groups per gender were compared using the Kruskal–Wallis test with post-hoc analysis to determine which groups were different. Correlations were analysed using Spearman's rho. A multiple regression analysis was performed using CIMT as the dependent variable, and age, body mass index, hip circumference, systolic and diastolic blood pressure and total cholesterol as independent variables. Because there were few male weight losers and extreme weight gainers, a sensitivity analysis was performed eliminating these participants (either or both groups).

Results

We initially recruited 180 participants. Of these, 20 had to be excluded because they did not comply with the inclusion criteria, ten did not have an objective record of their weight 10 years ago or failed to provide a full body photograph of the same period, and two subjects had high thyroid-stimulating hormone values on laboratory assessment. Therefore, the analyses were performed in 148 participants aged 42 (20–59) years (101 females). Data for male and female participants are shown in Table 1. Apart from the obvious gender differences in body composition and body mass index, it is noteworthy that females had a higher T/S ratio, high-density lipoprotein cholesterol and serum thyroxine.

Seventeen females were weight losers, 41 were weight maintainers, 15 were weight gainers and 28 were extreme weight gainers. Seven males were weight losers, 24 were weight maintainers, ten were weight gainers and six were extreme weight gainers. Data for females and males according to weight change are shown in Tables 2 and 3, respectively. Female weight gainers had a higher body mass index, waist circumference and total body fat. Only hip circumference and total fat mass was higher among male weight gainers. No differences were observed with respect to physical activity levels. Female weight losers and maintainers had a significantly lower HOMA index than female weight gainers. No other significant differences were observed between weight gainers and weight maintainers. Among males, the sensitivity analysis removing extreme groups was found to eliminate the differences in age, hip circumference and total fat mass generated by the group of extreme weight gainers, although no other changes in results were observed.

On a secondary analysis, the different measures of CIMT had a significant correlation with age, body mass index, hip circumference, systolic and diastolic blood pressure, and total cholesterol (Table 4). On a multiple regression analysis, only age remained as an independent predictor of left average, right average, left maximal and right maximal intima media thickness (Table 5). No correlation was observed between the T/S ratio and any other measured parameter, including age. If, on a

Table 1 Clinical and laboratory features of participants. Expressed as median (range)

	Females (n = 101)	Males (n = 47)	P*
Age (years)	42 (29 to 60)	42 (29 to 55)	
Body mass index (kg m^{-2})	24.7 (18 to 44.5)	26.9 (21.1 to 36.7)	<0.01
Waist circumference (cm)	87 (66 to 123.5)	95 (83.5 to 126)	<0.01
Hip circumference (cm)	99.5 (84 to 132)	101 (92 to 120)	
Wait/hip ratio	0.87 (0.77 to 1)	0.95 (0.85 to 1.1)	<0.01
Weight change over 10 years (kg)	4.5 (−8.9 to 22.7)	4.9 (−17.4 to 33.7)	
Weight change over 10 years (%)	7.8 (−14.1 to 42)	6.3 (−14.5 to 48.1)	
Energy intake (kJ day^{-1})	7597 (2097 to 19220)	8587 (2635 to 19830)	0.02
Protein intake (g day^{-1})	81.3 (20.1 to 226.9)	93.3 (21.6 to 228)	<0.01
Lipid intake (g day^{-1})	72 (15.5 to 213.2)	79.2 (27.7 to 302.7)	<0.01
Carbohydrate intake (g day^{-1})	191.2 (62.2 to 533.9)	259.1 (56.6 to 619.1)	<0.01
Systolic blood pressure (mmHg)	114 (91 to 149)	121 (101 to 186)	<0.01
Diastolic blood pressure (mmHg)	73 (54 to 100)	73 (56 to 107)	
Physical activity (METS min^{-1} $week^{-1}$)	727.5 (0 to 14 052)	1207.5 (0 to 19 066.5)	<0.01
Dual-energy X-ray absorptiometry			
Total bone mass (kg)	2.2 (1.3 to 3)	3.1 (2.2 to 4.2)	<0.01
Total fat mass (kg)	24.3 (12.3 to 49.4)	24.3 (13.2 to 47)	<0.01
Total fat free mass (kg)	37.7 (25.3 to 49)	55.7 (43.1 to 70.3)	
Carotid intima media thickness			
Average left (mm)	0.53 (0.37 to 0.84)	0.53 (0.32 to 0.88)	
Maximal left (mm)	0.6 (0.4 to 1.23)	0.6 (0.36 to 1)	
Average right (mm)	0.52 (0.24 to 1)	0.52 (0.39 to 1.04)	
Maximal right (mm)	0.59 (0.28 to 1.07)	0.6 (0.4 to 1.14)	
Laboratory values			
Blood glucose (mM)	4.7 (3.8 to 6.3)	4.9 (3.6 to 7.4)	
Total cholesterol (mM)	5.2 (3 to 8.7)	5.1 (3.2 to 8.4)	
HDL choleterol (mM)	1.5 (0.8 to 2.7)	1.1 (0.7 to 2.1)	<0.01
LDL cholesterol (mM)	2.9 (1.3 to 6.4)	3.1 (1.8 to 5.8)	
Triacylglycerol (mM)	1.2 (0.4 to 4)	1.4 (0.6 to 8)	
TSH (μU mL^{-1})	2.4 (0.6 to 10.1)	2.1 (0.5 to 10.8)	
Thyroxine (nM)	100.3 (66.9 to 177.1)	89.4 (66.5 to 129.1)	<0.01
Triiodothyronine (nM)	0.2 (0.1 to 0.3)	0.2 (0.1 to 0.3)	
Insulin (pM)	59 (15.3 to 330.6)	48.6 (18.1 to 320.9)	
HOMA-IR	1.7 (0.5 to 11.7)	1.5 (0.5 to 15.1)	
T/S ratio	1.5 (0.3 to 3)	1 (0.3 to 4.2)	<0.01
SIRT1 (ratio)	1 (0 to 141.4)	0.9 (0.5 to 57.5)	
SIRT6 (ratio)	0.9 (0.1 to 21.5)	0.8 (0.2 to 16.3)	

*Probability for differences between groups (Kruskal–Wallis).

Data are expressed as the median (range).

HDL, high-density lipoprotein; LDL, low-density lipoprotein; HOMA, homeostatic model assessment; METS, metabolic equivalents; T/S, ratio between telomeres and a housekeeping gene; TSH, thyroid-stimulating hormone.

sensitivity analysis, only weight losers and extreme weight gainers were compared or these extreme groups were removed from the analysis, the lack of difference in T/S ratio still persisted.

Discussion

The present study aimed to determine whether a history of weight gain over a period of 10 years is associated with a higher speed of biological ageing. We chose two main parameters to evaluate ageing: namely telomere length measured in PBMC and CIMT as an expression of vascular ageing. Sirtuin 1 and 6 expression in PBMC was used as a possible target of energy restriction. No differences in these markers were observed between weight gainers and weight maintainers. Among participants, there was a group of weight losers. We discarded the possibility that the weight reduction observed in these individuals was the result of an underlying disease because we took care to select healthy people for inclusion in the study.

In rodents, reducing energy intake by 25–50% invariably increases longevity (Fontana & Klein, 2007). In non-human primates, the results are not so straight forward. Two parallel studies were conducted and one of them

Table 2 Clinical and laboratory features of female participants according to weight change

	A: Weight losers (n = 17)	B: Weight maintainers (n = 41)	C: Weight gainers (n = 15)	D: Extreme weight gainers (n = 28)	P*
Age (years)	46 (29 to 56)	42 (30 to 60)	40 (29 to 55)	39 (29 to 55)	
Body mass index (kg m^{-2})	23.2 (18.2 to 31.4)	23.9 (18 to 38.6)	24.7 (21.6 to 44.5)	26.6 (21.9 to 36.5)	D versus A, B
Waist circumference (cm)	80 (66 to 100)	84 (67 to 112)	87 (80 to 123.5)	91.5 (75.2 to 110)	A versus C; A versus D
Hip circumference (cm)	96 (86 to 112)	97.5 (84 to 120)	100 (90 to 132)	102.5 (90 to 119)	A versus C, D; B versus C, D
Waist/hip ratio	0.86 (0.77 to 0.94)	0.87 (0.77 to 0.96)	0.88 (0.79 to 0.95)	0.88 (0.79 to 1.01)	
Weight change over 10 years (kg)	−2.6 (−8.9 to 0.2)	2.8 (0 to 7.6)	7.4 (5.3 to 11.4)	13 (7 to 22.7)	
Weight change over 10 years (%)	−4.2 (−14.1 to 0.3)	4.8 (0 to 9.8)	12.3 (10.2 to 15.2)	23.5 (16.7 to 42)	
Energy intake (kJ day^{-1})	5889 (3749 to 10210)	8172 (3036 to 12643)	6507 (5017 to 12280)	7539 (2097 to 19220)	
Protein intake (g day^{-1})	81 (29 to 142)	82 (38 to 138)	74 (52 to 227)	75 (20 to 183)	
Lipid intake (g day^{-1})	60 (32 to 104)	75 (27 to 169)	72 (33 to 165)	60 (16 to 213)	
Carbohydrate intake (g day^{-1})	191 (72 to 311)	195 (71 to 412)	178 (111 to 364)	220 (62 to 534)	
Systolic blood pressure (mmHg)	114 (93 to 137)	111 (91 to 147)	113 (102 to 149)	115 (100 to 142)	
Diastolic blood pressure (mmHg)	78 (60 to 92)	71 (54 to 94)	72 (63 to 91)	76 (61 to 100)	
Physical activity (METS min^{-1} $week^{-1}$)	805.5 (0 to 9375)	540 (0 to 14 052)	756 (0 to 1908)	1104.5 (0 to 4194)	
Dual-energy X-ray absorptiometry					
Total bone mass (kg)	2.1 (1.8 to 2.8)	2.2 (1.7 to 3)	2.2 (1.3 to 2.9)	2.3 (1.8 to 2.7)	
Total fat mass (kg)	20.7 (12.9 to 35.6)	23.4 (12.3 to 41.3)	24.3 (16.1 to 49.4)	29.3 (17.4 to 40.9)	D versus A, B; C versus A
Total fat free mass (kg)	36.6 (29.8 to 45.4)	36.3 (28.1 to 49)	38.3 (25.3 to 44.7)	38.2 (30.1 to 47.3)	
Carotid intima media thickness					
Average left (mm)	0.54 (0.37 to 0.8)	0.55 (0.37 to 0.75)	0.47 (0.42 to 0.65)	0.5 (0.38 to 0.84)	
Maximal left (mm)	0.56 (0.43 to 0.87)	0.6 (0.4 to 1.23)	0.56 (0.44 to 0.8)	0.58 (0.44 to 0.91)	
Average right (mm)	0.55 (0.38 to 0.84)	0.51 (0.35 to 0.79)	0.49 (0.43 to 0.86)	0.53 (0.24 to 1)	
Maximal right (mm)	0.6 (0.44 to 0.88)	0.59 (0.4 to 0.88)	0.56 (0.48 to 0.88)	0.62 (0.28 to 1.07)	
Laboratory values					
Blood glucose (mM)	4.7 (3.9 to 6.1)	4.7 (3.8 to 6.3)	4.7 (4 to 5.6)	4.9 (3.9 to 5.9)	
Total cholesterol (mM)	5.2 (3.7 to 6.9)	5.2 (3.3 to 7.3)	5.3 (3 to 8.7)	4.8 (3.9 to 6.4)	
HDL choleterol (mM)	1.8 (1 to 2.7)	1.5 (0.8 to 2.3)	1.6 (0.9 to 2.6)	1.4 (0.9 to 2.4)	
LDL cholesterol (mM)	2.8 (1.3 to 4.3)	3 (1.5 to 4.9)	3 (1.4 to 6.4)	2.8 (1.6 to 4.3)	
Triacylglycerol (mM)	1 (0.5 to 3)	1.2 (0.4 to 4)	1.1 (0.6 to 2.2)	1.3 (0.7 to 3.2)	
TSH (μU mL^{-1})	1.8 (0.6 to 5.2)	2.2 (0.8 to 9.7)	2.5 (1 to 4.2)	2.8 (0.7 to 10.1)	
Thyroxine (nM)	117 (66.9 to 177.1)	99.2 (73.7 to 146.2)	113.3 (81.3 to 147.2)	98.9 (68.3 to 169.8)	
Triiodothyronine (nM)	0.2 (0.1 to 0.3)	0.2 (0.1 to 0.3)	0.2 (0.1 to 0.3)	0.2 (0.1 to 0.3)	
Insulin (pM)	45.8 (26.4 to 115.3)	45.8 (15.3 to 155.6)	59 (33.3 to 330.6)	82.6 (32.6 to 255.6)	
HOMA-IR	1.3 (0.8 to 4.5)	1.4 (0.5 to 4.6)	1.8 (1 to 11.7)	2.6 (0.9 to 9.2)	A versus C, D; B versus D
T/S ratio	1.6 (0.8 to 2.5)	1.5 (0.4 to 2.9)	1.7 (0.3 to 2.2)	1.3 (0.6 to 3)	
SIRT1 (ratio)	1.2 (0 to 32.8)	0.9 (0.2 to 141.4)	1 (0.6 to 15.2)	1 (0.2 to 8.5)	
SIRT6 (ratio)	1 (0.4 to 2.4)	0.9 (0.1 to 13.3)	0.8 (0.5 to 15.8)	1.4 (0.5 to 21.5)	

*Significant differences between groups (Kruskal–Wallis, $P < 0.05$ or less).

Data are expressed as the median (range).

HDL, high-density lipoprotein; LDL, low-density lipoprotein; HOMA, homeostatic model assessment; METS, metabolic equivalents; T/S, ratio between telomeres and a housekeeping gene; TSH, thyroid-stimulating hormone.

Table 3 Clinical and laboratory features of male participants according to weight change. Expressed as median (range)

	A: Weight losers (n = 7)	B: Weight maintainers (n = 24)	C: Weight gainers (n = 10)	D: Extreme weight gainers (n = 6)	P*
Age (years)	45 (30 to 54)	40.5 (29 to 55)	45 (37 to 55)	37 (30 to 42)	D versus A, B, C
Body mass index (kg m^{-2})	27.9 (24.7 to 36.7)	26.5 (21.1 to 34.2)	27.4 (23.7 to 33.6)	28.1 (22.2 to 34.3)	
Waist circumference (cm)	98 (83.5 to 126)	92.3 (85 to 112)	100.4 (84.5 to 110)	102.8 (93.5 to 114)	
Hip circumference (cm)	103 (92.5 to 120)	98.5 (92.5 to 117)	100.5 (92 to 107)	106 (102 to 110)	D versus B, C
Waist/hip ratio	0.96 (0.85 to 1.1)	0.94 (0.86 to 1.02)	0.96 (0.89 to 1.1)	0.96 (0.91 to 1.0)	
Weight change in 10 years (kg)	−5.4 (−17.4 to 2.3)	3.8 (0 to 6.3)	8.9 (6.7 to 10.5)	15 (12.8 to 33.7)	
Weight change in 10 years (%)	−7 (−14.5 to 2.8)	5.2 (0 to 8.6)	11.4 (10.1 to 15.9)	20.3 (17 to 48.1)	
Energy intake (kJ day^{-1})	8342 (2635 to 15500)	8182 (3849 to 15959)	8775 (5542 to 19082)	11846 (6040 to 19830)	
Protein intake (g day^{-1})	88 (22 to 228)	89 (45 to 141)	101 (57 to 205)	137 (69 to 224)	
Lipid intake (g day^{-1})	74 (28 to 170)	73 (37 to 185)	91 (36 to 303)	139 (70 to 215)	
Carbohydrate intake (g day^{-1})	254 (74 to 446)	250 (57 to 456)	284 (131 to 619)	259 (124 to 546)	
Systolic blood pressure (mmHg)	120 (106 to 186)	123.5 (101 to 141)	117.5 (106 to 130)	123.5 (113 to 137)	
Diastolic blood pressure (mmHg)	77 (56 to 107)	71 (60 to 88)	72 (64 to 80)	73 (66 to 81)	
Physical activity (METS min^{-1} week^{-1})	906.5 (0 to 4005)	1059 (0 to 3386)	2090.3 (99 to 19 066.5)	2093.3 (0 to 6930)	
Dual-energy X-ray absorptiometry					
Total bone mass (kg)	2.8 (2.7 to 3.4)	3.1 (2.3 to 4.2)	3.3 (2.2 to 3.4)	3 (2.9 to 3.5)	
Total fat mass (kg)	25.8 (15.1 to 47)	21.8 (13.2 to 37.3)	24.3 (16.4 to 33.3)	30.2 (25.2 to 35.9)	D versus A, B
Total fat free mass (kg)	55.2 (51.2 to 70.3)	53 (46.2 to 68.4)	57.2 (43.1 to 64.2)	56.6 (54.2 to 67.8)	
Carotid intima media thickness					
Average left (mm)	0.58 (0.47 to 0.76)	0.53 (0.32 to 0.88)	0.51 (0.45 to 0.65)	0.51 (0.42 to 0.64)	
Maximal left (mm)	0.6 (0.52 to 0.8)	0.6 (0.36 to 1)	0.6 (0.48 to 0.76)	0.58 (0.4 to 0.82)	
Average right (mm)	0.57 (0.42 to 1.04)	0.53 (0.4 to 0.88)	0.51 (0.4 to 0.67)	0.47 (0.39 to 0.76)	
Maximal right (mm)	0.67 (0.44 to 1.14)	0.62 (0.4 to 0.96)	0.56 (0.48 to 0.76)	0.54 (0.44 to 0.88)	
Laboratory values					
Blood glucose (mM)	4.9 (4.3 to 7.4)	4.9 (3.6 to 6)	5.1 (4.3 to 6)	5.1 (4.7 to 6.5)	
Total cholesterol (mM)	5.4 (4.7 to 5.7)	4.9 (3.2 to 7.5)	5.6 (4.5 to 7.5)	4.8 (4.1 to 8.4)	
HDL choleterol (mM)	1.2 (1 to 2.1)	1.1 (0.8 to 1.8)	1 (0.7 to 1.6)	1 (0.9 to 1.2)	
LDL cholesterol (mM)	3.2 (2.9 to 3.8)	2.9 (1.8 to 5.8)	3.6 (2 to 5.1)	2.9 (2.3 to 3.5)	
Triacylglycerol (mM)	1.5 (0.7 to 2.2)	1.3 (0.6 to 3.4)	1.4 (0.8 to 6.6)	2.3 (0.7 to 8)	
TSH (μU mL^{-1})	3.1 (1.6 to 4.7)	2.1 (0.9 to 10.8)	1.7 (1 to 4.2)	1.9 (0.5 to 8.4)	
Thyroxine (nM)	90.1 (71.8 to 95.6)	86.7 (66.5 to 111.3)	103.7 (68.6 to 129.1)	89.4 (83.1 to 109)	
Triiodothyronine (nM)	0.2 (0.2 to 0.2)	0.2 (0.1 to 0.2)	0.2 (0.2 to 0.2)	0.2 (0.2 to 0.3)	
Insulin (pM)	69.5 (33.3 to 320.9)	45.8 (22.9 to 114.6)	52.4 (18.1 to 184)	74.3 (37.5 to 301.4)	
HOMA-IR	2.2 (1 to 15.1)	1.4 (0.7 to 3.4)	1.8 (0.5 to 6)	2.5 (1.2 to 9.3)	
T/S ratio	0.7 (0.3 to 1.4)	1.1 (0.5 to 4.2)	1 (0.8 to 1.8)	0.9 (0.3 to 2.7)	
SIRT1 (ratio)	1 (0.7 to 1.9)	0.9 (0.6 to 57.5)	1.2 (0.6 to 4.9)	0.8 (0.5 to 1)	
SIRT6 (ratio)	0.9 (0.3 to 3.3)	1 (0.3 to 16.3)	1.1 (0.2 to 5.2)	0.5 (0.2 to 0.8)	

*Significant differences between groups (Kruskal Wallis, $P < 0.05$ or less).

Data are expressed as the median (range).

HDL, high-density lipoprotein; LDL, low-density lipoprotein; HOMA, homeostatic model assessment; METS, metabolic equivalents; T/S, ratio between telomeres and a housekeeping gene; TSH, thyroid-stimulating hormone.

showed an increase in longevity (Colman *et al.*, 2009), whereas the other did not find any change in this parameter (Mattison *et al.*, 2012). However, both studies observed a reduction in the incidence of ageing-related conditions, such as cardiovascular disease and tumours. Performing energy restriction interventions in humans is much more complicated. The most successful attempt was the CALERIE study, carried out over 6 months.

Table 4 Correlation matrix for measures of carotid intima media thickness

	Carotid intima media thickness			
	Left average	Right average	Left maximal	Right maximal
Age (years)	0.43*	0.44	0.47	0.43
	0.00†	0.00	0.00	0.00
Body mass index (kg m^{-2})	0.05	0.22	0.03	0.24
	0.58	0.01	0.71	0.01
Waist circumference (cm)	0.01	0.14	0.03	0.14
	0.90	0.14	0.75	0.12
Hip circumference (cm)	0.06	0.24	0.06	0.23
	0.49	0.01	0.49	0.01
Total body fat (kg)	0.01	0.14	−0.01	0.15
	0.92	0.12	0.92	0.10
Total fat free mass (kg)	0.11	0.15	0.08	0.15
	0.25	0.09	0.38	0.11
Systolic blood pressure (mmHg)	0.17	0.26	0.19	0.27
	0.07	0.00	0.04	0.00
Diastolic blood pressure (mmHg)	0.22	0.31	0.22	0.31
	0.02	0.00	0.02	0.00
Total cholesterol (mM)	0.22	0.19	0.22	0.18
	0.02	0.04	0.02	0.05
HDL cholesterol (mM)	−0.01	−0.04	0.03	−0.05
	0.90	0.67	0.73	0.57
LDL cholesterol (mM)	0.19	0.14	0.19	0.13
	0.04	0.13	0.04	0.14
Triacylglycerol (mM)	0.13	0.17	0.09	0.17
	0.17	0.07	0.33	0.06
Homa-IR (arbitrary units)	−0.07	0.04	−0.07	0.06
	0.45	0.70	0.48	0.52
T/S ratio	0.00	0.07	0.01	0.04
	0.96	0.44	0.87	0.67

*Spearman's rho.
†Probability.
HDL, high-density lipoprotein; LDL, low-density lipoprotein; HOMA, homeostatic model assessment; T/S, ratio between telomeres and a housekeeping gene.

Among participants with a reduced energy intake, core temperature, insulin and triiodothyronine levels decreased (Heilbronn *et al.*, 2006). Also, a reduction in carbonyl levels was observed (Meydani *et al.*, 2011). These experiments demonstrate that, with a great effort, energy restriction interventions are feasible in humans, although they are extraordinarily expensive and the results are far from spectacular. Indeed, the same authors are carrying out a 2-year intervention trial in an effort to obtain clearer cut outcomes (Rochon *et al.*, 2011).

The main explanation for the limited conclusions that can be drawn from these experiments is the relatively short period during which ER is carried out. The intervention in rodents and nonhuman primates lasts the whole of the adult lifetime of the animals. In humans, a 1-year trial would represent < 2% of their lifespan. Therefore, the search for other models of energy restriction to reproduce the results obtained in experimental animals is crucial. One such model is a retrospective assessment of energy intake using weight stability that is similar to the primate model used by Bodkin *et al.* (2003) in which energy restriction was titrated to maintain a normal weight. If an individual maintains a stable weight during a certain lapse, the assumption is that he should be in energy balance. In other words, energy expenditure should be equal to energy intake. There are two drawbacks to this model. The first and most important is to have an objective recording of weight, ≥10 years back in time. Clearly, the sole recall of a former weight by participants is highly inaccurate and unacceptable. If there is any form of objective record of weight, this problem is solved. The second limitation is the real quantification of the effect of exercise on the maintenance of energy balance. This is a relatively minor problem, considering that most adults are sedentary, at least in Chile where <10% of the population performs more than 30 min of exercise per week, according to health status surveys (Ministerio de Salud, 2010). Excluding the minority of participants who perform more exercise is a simple way of eliminating the bias introduced by physical activity. However, exercise is a variable that must be held in mind. Recent reports show that the exercising muscle secretes a hormone that could prevent some of the consequences of ageing (Boström *et al.*, 2012). In the present investigation, we only recruited participants with objective information about their weight 10 years ago. To ensure the validity of such information, we requested them to provide a photograph to visually corroborate the information. We also excluded subjects that used to exercise frequently. Therefore, we took care of the main limitations of the model.

The second problem regarding studies performed in humans is the choice of adequate markers of ageing. Even in retrospective studies, it is impossible to evaluate changes in mortality or incidence of age-related diseases.

Measurement of telomere length has become popular ever since rather simple PCR technique to measure it was reported (Cawthon, 2002). In the ensuing years, reports showed that telomeres of genomic DNA of PBMC shortened with age and also under situations or conditions associated with a shorter lifespan, such as obesity, chronic stress or smoking (Cawthon *et al.*, 2003; Valdes *et al.*, 2005). On the other side of the spectrum, centenary Askenazi Jews have longer telomeres (Atzmon *et al.*, 2010). The problem with this marker is that its measurement in one type of cell is a huge extrapolation from what happens in the whole multicellular organism. The

Table 5 Multiple linear regression models for the different measures of carotid intima media thickness

	Left average R^2 = 0.25			Right average R^2 = 0.23			Left maximal R^2 = 0.22			Right maximal R^2 = 0.20		
	Coefficient†	SE	P‡	Coefficient	SE	P	Coefficient	SE	P	Coefficient	SE	P
Age	0.006	0.001	<0.01	0.004	0.001	<0.01	0.007	0.001	<0.01	0.005	0.001	<0.01
Body mass index	−0.003	0.004	0.434	−0.003	0.005	0.543	−0.004	0.004	0.432	0.000	0.005	0.929
Hip circumference	0.002	0.002	0.315	0.004	0.003	0.119	0.001	0.002	0.646	0.003	0.003	0.228
Systolic blood pressure*	0.000	0.001	0.958	0.001	0.001	0.332	0.000	0.001	0.804	0.001	0.001	0.720
Diastolic blood pressure*	0.001	0.002	0.440	0.002	0.002	0.372	0.000	0.002	0.811	0.002	0.002	0.376
Total cholesterol	0.000	0.000	0.948	0.000	0.000	0.780	0.000	0.000	0.919	0.000	0.000	0.835
Constant	0.074	0.151	0.624	−0.239	0.192	0.214	0.234	0.179	0.193	−0.099	0.205	0.631

*Because systolic and diastolic blood pressure values were highly correlated, their possible weight on the dependent variable was estimated on separate models.

†Estimated coefficient of the model.

‡Probability for t statistic.

other problem is the large dispersion of values that we and others have observed (Bunout *et al.*, 2009). Even considering these limitations, telomere length is a useful marker of ageing. In the present study, we observed that men had a lower T/S ratio than women, although no differences in age between genders were observed. This finding has been reported previously (Benetos *et al.*, 2001) and may be related to the shorter lifespan of men than women. As a weakness of the present study, we did not observe a correlation between telomere length and age. However, this was expected, considering that we studied participants of an age range during which there is little variation in telomere length (Aubert & Lansdorp, 2008). Another possibility is that an observation period larger than 10 years is required to observe differences between weight maintainers and gainers. Finally, the sample size could also be a limitation with respect to to observing differences in telomere length.

We also measured the expression of Sirtuin 1 and 6 in PBMC. Both are NAD-dependent deacetylases, which have a series of metabolic actions such as DNA repair and mitochondrial biogenesis that should avoid the deleterious effects of ageing (Guarente, 2008). In humans, a short energy restriction period was associated with an up-regulation of Sirtuin 1 expression (Crujeiras *et al.*, 2008; Kitada *et al.*, 2013). Therefore, we also measured these enzymes without finding differences between subjects that maintained or gained weight. We did not attempt to separate subpopulations of PBMC to measure telomeres or sirtuin 1 and 6 activities because we would have to withdraw much larger blood samples from participants to obtain an adequate amount of genomic material, especially mRNA to perform the measurements.

Carotid intima media thickness measurement is a simple and non-invasive means of evaluating vascular damage, which is directly related to cardiovascular risk factors (Herder *et al.*, 2012). However, age is one of the main independent determinants of this parameter (Khalil *et al.*, 2010; Su *et al.*, 2012). We have recently demonstrated that this parameter is associated with age and disability in older people (Barrera *et al.*, 2013). Therefore, it is reasonable to use it as a marker of vascular ageing. In the present study, we did not observe any difference between weight maintainers and weight gainers. However, as expected, CIMT was associated with age.

In conclusion, we were unable to show a difference in telomere length or CIMT between subjects who maintained a stable weight and those who gained weight over a period of 10 years. As a future direction, these participants should be assessed in the next 5–10 years to observe eventual changes in the measured parameters. The retrospective model proposed probably cannot be used as a proxy of energy restriction in humans.

Conflict of interests, source of funding and authorship

The authors declare that they have no conflict of interests.

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DB, GB and SH conceived and designed the study; generated, collected, assembled, analysed and/or interpreted data; and drafted or revised the manuscript. MPM conceived and designed the study and drafted or revised the manuscript. LL generated, collected, assembled, analysed and/or interpreted data. All authors critically reviewed the manuscript and approved the final version submitted for publication.

References

Atzmon, G., Cho, M., Cawthon, R.M., Budagov, T., Katz, M., Yang, X., Siegel, G., Bergman, A., Huffman, D.M., Schechter, C.B., Wright, W.E., Shay, J.W., Barzilai, N., Govindaraju, D.R. & Suh, Y. (2010) Evolution in health and medicine Sackler colloquium: genetic variation in human telomerase is associated with telomere length in Ashkenazi centenarians. *Proc. Natl Acad. Sci. USA* **107**(Suppl. 1), 1710–7.

Aubert, G. & Lansdorp, P.M. (2008) Telomeres and aging. *Physiol. Rev.* **88**, 557–79.

Barrera, G., Bunout, D., de la Maza, M.P., Leiva, L. & Hirsch, S. (2013) Carotid ultrasound examination as an aging and disability marker. *Geriatr. Gerontol. Int.* doi: 10.1111/ggi.12146 [Epub ahead of print].

Benetos, A., Okuda, K., Lajemi, M., Kimura, M., Thomas, F., Skurnick, J., Labat, C., Bean, K. & Aviv, A. (2001) Telomere length as an indicator of biological aging: the gender effect and relation with pulse pressure and pulse wave velocity. *Hypertension* **37**, 381–5.

Bodkin, N.L., Alexander, T.M., Ortmeyer, H.K., Johnson, E. & Hansen, B.C. (2003) Mortality and morbidity in laboratory-maintained Rhesus monkeys and effects of long-term dietary restriction. *J. Gerontol. A Biol. Sci. Med. Sci.* **58**, 212–9.

Boström, P., Wu, J., Jedrychowski, M.P., Korde, A., Ye, L., Lo, J.C., Rasbach, K.A., Boström, E.A., Choi, J.H., Long, J.Z., Kajimura, S., Zingaretti, M.C., Vind, B.F., Tu, H., Cinti, S., Højlund, K., Gygi, S.P. & Spiegelman, B.M. (2012) A PGC1-α-dependent myokine that drives brown-fat-like development of white fat and thermogenesis. *Nature* **481**, 463–8.

Bunout, D., Backhouse, C., Leiva, L., Barrera, G., Sierralta, W., de la Maza, M.P., Hirsch, S. (2009) Relationship between protein and mitochondrial DNA oxidative injury and telomere length and muscle loss in healthy elderly subjects. *Arch. Gerontol. Geriatr.* **48**, 335–9.

Cardus, A., Uryga, A.K., Walters, G. & Erusalimsky, J.D. (2013) SIRT6 protects human endothelial cells from DNA damage, telomere dysfunction, and senescence. *Cardiovasc. Res.* **97**, 571–9.

Cawthon, R. (2002) Telomere measurement by quantitative PCR. *Nucleic Acids Res.* **30**, e47.

Cawthon, R.M., Smith, K.R., O'Brien, E., Sivatchenko, A. & Kerber, R.A. (2003) Association between telomere length in blood and mortality in people aged 60 years or older. *Lancet* **361**, 393–5.

Colman, R.J., Anderson, R.M., Johnson, S.C., Kastman, E.K., Kosmatka, K.J., Beasley, T.M., Allison, D.B., Cruzen, C., Simmons, H.A., Kemnitz, J.W. & Weindruch, R. (2009) Caloric restriction delays disease onset and mortality in rhesus monkeys. *Science* **325**, 201–4.

Crujeiras, A.B., Parra, D., Goyenechea, E. & Martínez, J.A. (2008) Sirtuin gene expression in human mononuclear cells is modulated by caloric restriction. *Eur. J. Clin. Invest.* **38**, 672–8.

De la Maza, M.P., Uribarri, J., Olivares, D., Hirsch, S., Leiva, L., Barrera, G. & Bunout, D. (2008) Weight increase is associated with skeletal muscle immunostaining for advanced glycation end products, receptor for advanced glycation end products, and oxidation injury. *Rejuvenation Res.* **11**, 1041–8.

Fontana, L. & Klein, S. (2007) Aging, adiposity, and calorie restriction. *JAMA*. **297**, 986–94.

Fontana, L., Meyer, T.E., Klein, S. & Holloszy, J.O. (2004) Long-term calorie restriction is highly effective in reducing the risk for atherosclerosis in humans. *Proc. Natl Acad. Sci. USA* **101**, 6659–63.

Guarente, L. (2008) Mitochondria–a nexus for aging, calorie restriction, and sirtuins? *Cell* **132**, 171–6.

Heilbronn, L.K., de Jonge, L., Frisard, M.I., DeLany, J.P., Larson-Meyer, D.E., Rood, J., Nguyen, T., Martin, C.K., Volaufova, J., Most, M.M., Greenway, F.L., Smith, S.R., Deutsch, W.A., Williamson, D.A. & Ravussin, E.; Pennington CALERIE Team. (2006) Effect of 6-month calorie restriction on biomarkers of longevity, metabolic adaptation, and oxidative stress in overweight individuals: a randomized controlled trial. *JAMA*. **295**, 1539–48.

Herder, M., Johnsen, S.H., Arntzen, K.A. & Mathiesen, E.B. (2012) Risk factors for progression of carotid intima-media thickness and total plaque area: a 13-year follow-up study: the Tromsø Study. *Stroke* **43**, 1818–23.

Khalil, Y., Mukete, B., Durkin, M.J., Coccia, J. & Matsumura, M.E. (2010) A comparison of assessment of coronary calcium vs carotid intima media thickness for determination of vascular age and adjustment of the Framingham Risk Score. *Prev. Cardiol.* **13**, 117–21.

Kim, Y., Park, I. & Kang, M. (2013) Convergent validity of the International Physical Activity Questionnaire (IPAQ): meta-analysis. *Public Health Nutr.* **16**, 440–52.

Kitada, M., Kume, S., Takeda-Watanabe, A., Tsuda, S., Kanasaki, K. & Koya, D. (2013) Calorie restriction in overweight males ameliorates obesity-related metabolic alterations and cellular adaptations through anti-aging effects, possibly including AMPK and SIRT1 activation. *Biochim. Biophys. Acta* **1830**, 4820–7.

Matthews, D.R., Hosker, J.P., Rudenski, A.S., Naylor, B.A., Treacher, D.F. & Turner, R.C. (1985) Homeostasis model assessment: insulin resistance and beta-cell function from fasting plasma glucose and insulin concentrations in man. *Diabetologia* **28**, 412–9.

Mattison, J.A., Roth, G.S., Beasley, T.M., Tilmont, E.M., Handy, A.M., Herbert, R.L., Longo, D.L., Allison, D.B., Young, J.E., Bryant, M., Barnard, D., Ward, W.F., Qi, W., Ingram, D.K. & de Cabo, R. (2012) Impact of caloric restriction on health and survival in rhesus monkeys from the NIA study. *Nature* **489**, 318–21.

Meydani, M., Das, S., Band, M., Epstein, S. & Roberts, S. (2011) The effect of caloric restriction and glycemic load

on measures of oxidative stress and antioxidants in humans: results from the CALERIE Trial of Human Caloric Restriction. *J. Nutr. Health Aging* **15**, 456–60.

Ministerio de Salud. (2010) Encuesta Nacional de Salud 2009-2010. Available at: http://www.minsal.gob.cl.

Rahman, M., Halade, G.V., Bhattacharya, A. & Fernandes, G. (2009) The fat-1 transgene in mice increases antioxidant potential, reduces pro-inflammatory cytokine levels, and enhances PPAR-gamma and SIRT-1 expression on a calorie restricted diet. *Oxid. Med. Cell. Longev.* **2**, 307–16.

Rochon, J., Bales, C.W., Ravussin, E., Redman, L.M., Holloszy, J.O., Racette, S.B., Roberts, S.B., Das, S.K., Romashkan, S., Galan, K.M., Hadley, E.C. & Kraus, W.E.; CALERIE Study Group. (2011) Design and conduct of the CALERIE study: comprehensive assessment of the long-term effects of reducing intake of energy. *J. Gerontol. A Biol. Sci. Med. Sci.* **66**, 97–108.

Seventh Report of the Joint National Committee on Prevention, Detection, Evaluation, and Treatment of High Blood Pressure (JNC 7 Express). (2013) Available at: http://www.nhlbi.nih.gov/guidelines/hypertension/jncintro.htm on February 13 2013.

Stein, J.H., Korcarz, C.E. & Post, W.S. (2009) Use of carotid ultrasound to identify subclinical vascular disease and evaluate cardiovascular disease risk: summary and discussion of the American Society of Echocardiography consensus statement. *Prev. Cardiol.* **12**, 34–8.

Su, T.C., Chien, K.L., Jeng, J.S., Chen, M.F., Hsu, H.C., Torng, P.L., Sung, F.C. & Lee, Y.T. (2012) Age- and gender-associated determinants of carotid intima-media thickness: a community-based study. *J. Atheroscler. Thromb.* **19**, 872–80.

Valdes, A.M., Andrew, T., Gardner, J.P., Kimura, M., Oelsner, E., Cherkas, L.F., Aviv, A. & Spector, T.D. (2005) Obesity, cigarette smoking, and telomere length in women. *Lancet* **366**, 662–4.

Walford, R.L., Mock, D., Verdery, R. & MacCallum, T. (2002) Calorie restriction in biosphere 2: alterations in physiologic, hematologic, hormonal, and biochemical parameters in humans restricted for a 2-year period. *J. Gerontol. A Biol. Sci. Med. Sci.* **57**, B211–24.

Weindruch, R. & Sohal, R.S. (1997) Seminars in medicine of the Beth Israel Deaconess Medical Center. Caloric intake and aging. *N. Engl. J. Med.* **337**, 986–94.

Zhang, P., Li, H., Tan, X., Chen, L. & Wang, S. (2013) Association of metformin use with cancer incidence and mortality: a meta-analysis. *Cancer Epidemiol.* **2013**, 207–218.