Effect of epigallocatechin gallate on the body composition and lipid profile of Down syndrome individuals: implications for clinical management

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Summary

Background & Aims

Individuals with Down syndrome (DS) have higher rates of obesity. In the general population green tea extracts, and in particular epigallocatechin gallate (EGCG), have been studied for their antiobesogenic effects. The aim of this study is to elucidate the effect of EGCG on body weight in young DS adults and whether it could be related to changes in lipid profile.

Methods

In the context of a double-blind phase II clinical trial comparing the effect of EGCG to that of placebo, the body composition of 77 young adults with DS was analyzed through bioelectrical impedance analysis. Lipids were analyzed using standard laboratory procedures. The factors tested in the ANCOVA model for the differences from baseline were treatment, sex as well as their interaction as independent variables. Baseline values were included in the models as covariates.

Results

Individuals receiving placebo showed an increase in body weight and body mass index (BMI) that was not detected in those with EGCG treatment. EGCG effect on body composition was mainly observed in males, with significant differences between the EGCG and the placebo group after 12 months for weight (estimated adjusted mean difference (AMD)= -2.34, 95% CI=[-4.21, -0.48]; p=0.015) and body fat (estimated AMD=-1.23, 95% CI=[-2.43,-0.04], p=0.043). The changes detected in body composition were associated with changes in lipid profile.

Conclusions

Our results suggest that EGCG could have a modest beneficial effect on weight management in DS. Furthermore, EGCG has also a sex-dependent effect on lipid profile that is related to changes in body mass and composition.

Keywords: Down syndrome, epigallocatechin gallate, weight management, body fat, lipid profile

Introduction

It has long been acknowledged that the prevalence of obesity is greater in individuals with intellectual disability than in the general population [1, 2]. In the case of Down syndrome (DS), higher rates of obesity [3], starting from childhood [4], have been reported. Nevertheless, some studies argue that it is mainly DS females who have a higher body mass index (BMI) than controls [5]. The elevated rates of obesity in DS individuals are generally assumed to be related to their higher levels of inactivity and calorie intake [6], although another possible factor could be a greater prevalence of hypothyroidism [4]. Regarding the distribution of fat, children and adolescents with DS have been found to have different fat distributions than age-matched controls, in particular, females had higher fat mass in the trunk and lower in the lower body compared to controls, while males had higher total fat mass and trunk fat mass [7].

During the past decade, the health-promoting effects of green tea and its polyphenols have been extensively investigated. Among the postulated health benefits is body weight reduction, although the mechanisms involved have not yet been fully elucidated. However, EGCG and green tea have proven to decrease digestion and absorption of lipids, decrease the secretion of pancreatic lipase, as well as glucose absorption, all of which could lead to a decrease in body gain [8]. In clinical studies, epigallocatechin gallate (EGCG), the main catechin of green tea, has shown its potential to decrease body weight and fat, while increasing fat oxidation and thermogenesis [9]. Green tea has also proven to modify lipid profile, decreasing low density lipoprotein (LDLc) cholesterol, total cholesterol (TC) [10] and oxidized low density lipoproteins (oxLDL) [11] in experimental models and humans, being effective estimated doses 500 mg catechins (6-7 cups of tea). This modification of lipid profile has been established to be beneficial for the cardiovascular system [8]. In a recent clinical trial we reported that EGCG, in combination with cognitive training, improved cognitive function and adaptive functionality in young adults with DS [12]. The lipid profile of the participants was evaluated and we observed

differences among treatments for TC, HDLc, and oxLDL [12]. Interestingly, previous studies in adolescents detected a correlation between total body fat and lipid profile in males [13], and between lipid profile and BMI and body fat percentage in adult males and females [14].

Additionally, some epidemiological studies have shown that green tea could have a positive effect on bone mineral density, although the effects seem to vary between populations [15]. Similarly, clinical studies have failed to replicate those results [15]. Animal studies, specifically in DS have shown that the effects of EGCG on bone structure depend on dosage [16], with high dosages being detrimental [17]. In humans, a single study has looked into the effect of EGCG on the skeletal system, focusing on facial morphology, with the most relevant effects on the younger subjects (between 0 and 3) [18].

In this study we analyze the effects of EGCG on body composition, including bone mass, in DS, and whether they are sex-dependent and associated with changes in lipid profile.

Methods

Participants and treatment

Our cohort is a subsample of the TESDAD study [12], a 18-month, randomized, double-blind parallel placebo-controlled, therapeutic exploratory phase II clinical trial with young DS adults (ClinicalTrials.gov NCT01699711). It was conducted in accordance with the Declaration of Helsinki and Spanish laws concerning data privacy and approved by the local ethical committee (CEIC-PSMAR, Clinical research ethical committee of the Parc de Salut Mar). Individuals were recruited in the Hospital del Mar Medical Research Institute of Barcelona (Spain) where the study took place. Of the 87 individuals taking part, we included only those whose measurements regarding the variables of the present report were available at all time points. Three individuals who were under treatment with cholesterol lowering drugs were excluded from the analysis (n=77). The sample was randomized into two treatment conditions: one consisted of EGCG (9 mg/kg) and cognitive training, and another of placebo EGCG and cognitive training, as previously reported [12]. EGCG was administered in capsules of green tea extract (prepared ad-hoc by Life Extension for the study, www.lifeextension.com) containing 200 mg of EGCG. Placebo capsules were indistinguishable in appearance from EGCG capsules so volunteers, their families, and the evaluators were blinded as to treatment branch. Compliance was assessed by the return of the capsule bottles. Total daily doses administered of 400, 600 or 800mg depending on body weight, were distributed twice daily with meals (from 2 to 4 capsules per day). Selection of EGCG dosage was based on a pilot study designed at evaluating safety and preliminary efficacy of EGCG in the same population [19]. The intervention lasted for 12 months with a 6-month follow-up after treatment discontinuation.

Upon arrival at the Hospital del Mar Medical Research Institute, and prior to participating in the trial, participants, parents, and legal guardians were informed of the ensuing protocol and gave their written informed consent.

Body Mass measurements

Body composition (including weight, body fat, % of body fat, % of trunk body fat, % of lower body fat, and bone mass) was measured using a Tanita Multi-Frequency Body Composition Analyzer MC-180MA (Tanita Corporation, Tokyo, Japan), a validated instrument for bioelectrical impedance analysis (BIA) which uses low frequency electrical signals (1, 5, 50, 250, and 500 Khz) to calculate water and fat content. This gives two parameters: fat mass and lean mass, from the last one it estimates both skeletal musculature mass and bone mass. BIA and height were assessed without shoes and with minimum clothes, and all participants fasted overnight before measurements.

Plasma lipid measurements

Blood samples were collected early in the morning, after an overnight fast of 10-12 hours. Blood was drawn into 8mL Heparin Lithium tubes (B&D, UK), centrifuged at 4ºC for 15 min at 1700 g, and the plasma was distributed in aliquots and stored at -70ºC until analysis. Total cholesterol, triglyceride and HDL-cholesterol were determined by an accelerator selective detergent method (ABX-Horiba Diagnostics, Montpellier, France), in an automated PENTRA-400 analyzer (ABX-Horiba Diagnostics, Montpellier, France). Low density lipoprotein (LDL) cholesterol was calculated by the Friedewald formula whenever triglycerides were <300 mg/dL (LDLc = TC – (HDLc + TG/5) in mg/dL or LDLc= TC – (HDLc + TG/2.21) in mmol/L). Oxidized LDL (oxLDL) concentrations in plasma were measured by a sandwich ELISA procedure using the murine monoclonal antibody mAB-46 as the capture antibody bound to microtitration wells and a peroxidase conjugated anti-apolipoprotein B antibody that recognizes oxLDL bound to the solid phase (oxLDL, Mercodia, Uppsala, Sweden).

Statistical methods

Sample size was determined based on the primary objective of the TESDAD study [12] resulting in a total of 85 participants. A descriptive analysis of the variables of interest is presented using the mean, standard deviation, and range. ANCOVA models were used to explore the association between sex and body composition in basal conditions controlling for age and height.

For the longitudinal analysis of the differences from baseline during the study period (12 months), ANCOVA models were applied including treatment (EGCG or Placebo), sex, and basal values as independent variables as well as the interaction between treatment and sex. The effect size measure of interest was the adjusted mean difference between both treatments with respect to changes from baseline among both women and men. The same type of models was used to compare the differences after 6 months of treatment discontinuation to baseline for both treatments. The computation of simultaneous confidence intervals and adjusted p-values in order to guarantee a family-wise error rate of 0.05 was based on the multivariate t distribution of the vector of test statistics. The model assumptions, that is, homoscedasticity and normality, were graphically checked by means of residual plots.

Correlations between differences in body composition and in lipid profile were calculated through either Pearson or Spearman correlations after using the Lilliefors test to check for normality.

Statistical significance was set at 0.05. All statistical analyses were performed using the statistical software package R (Version 3.4.3.; The R Foundation for Statistical Computing, Vienna, Austria).

Results

Baseline body composition

Seventy-seven individuals (36 females, 47%) with a mean age (± standard deviation) of 23.4±4.3 years (range: 16-34) were included. Sex distribution according to treatment condition was as follows, Placebo: 18 males (49%) and 19 females (51%), EGCG 23 males (58%) and 17 females (42%). Underweight individuals accounted for 2.6% of the total, 44.2% were of normal weight, 37.7% were overweight, and 15.6% were obese. Baseline body composition descriptive measures are shown in Table 1. When adjusting for age and height (females were, on average, 10 cm shorter), females had a significantly higher body fat percentage, greater low body fat percentage, and lower bone mass. In addition, females showed a non-significant trend towards higher total body fat and lower trunk body fat percentage.

Table 1: Baseline body composition characteristics (including sex-stratification). Values are presented as means, standard deviations, and ranges. Statistics are presented as the estimated AMD between groups, standard error, and p-value.

	Total (n=77) Mean ± SD Range	**Males (n=41)** Mean ± SD Range	**Females (n=36)** Mean ± SD Range	**Estimated adjusted mean difference** **Standard Error** **p-value**

Height (cm)	153.6±9.0 134.0-171.0	158.8±7.9 146.0-171.0	147.7±6.2 134.0-162.5	**-10.985** **1.664** **5.35e^{-09}**
Weight (kg)	60.6±10.3 38.6-80.5	63.9±9.0 50.7-80.2	56.8±10.4 38.6-80.5	-3.670 2.759 0.18
BMI (kg/m^2)	25.8±4.3 17.9-41.9	25.4±3.6 19.9-35.1	26.1±5.0 17.9-41.9	-1.526 1.211 0.21
Body fat (kg)	11.32±6.0 1.5-29.1	9.4±5.4 2.1-24.0	13.5±6.1 1.5-29.1	*3.155* *1.223* *0.064*
% of body fat	18.2±7.9 3.7-36.1	14.1±6.7 3.7-29.9	22.7±6.7 3.8-36.1	**6.337** **1.936** **0.0016**
% of trunk fat	7.7±4.3 1.5-20.2	8.3±4.9 1.5-20.2	6.9±3.2 1.5-13.1	*-2.394* *1.223* *0.054*
% of lower body fat	8.3±4.9 1.4-19.0	4.2±1.7 1.4-9.2	13.1±2.3 5.7-19.0	**7.815** **0.562** **<2e^{-16}**
Bone mass (kg)	2.5±0.4 1.8-3.15	2.8±0.3 2.2-3.1	2.2±0.3 1.8-2.6	**-0.343** **0.065** **1.19e^{-06}**

Longitudinal effect of EGCG on body composition

Due to the differences observed between sexes in the basal data, the longitudinal analysis was performed stratifying by sex. As such, trends for differences were observed between

treatment groups in males after 12 months of treatment **(Table 2 and Figure 1)**. Whilst the mean values of weight and BMI increased over time in the placebo group, the EGCG treatment group did not change (**Figure 1**) over time. The trend for differences in weight between groups was maintained at 18 months, i.e. 6 months after treatment discontinuation **(Table 2 and Figure 2)**. In the case of females, no significant differences were observed at any time point for either BMI or weight, with the only trend being decreased bone mass at 12 months in the EGCG-treated group.

Table 2: Baseline body composition values and differences from baseline after 12 and 18 months (mean; standard deviation) according to treatment and gender.

		Placebo + CT			EGCG + CT			AMD (95%-CI; p-value)	
		Baseline (Females n=19, Males n=18)	12 months (Females n=19, Males n=18)	18 months (Females n=19, Males n=18)	Baseline (Females n=17, Males n=23)	12 months (Females n=17, Males n=23)	18 months (Females n=17, Males n=23)	After 12 months[1]	After 18 months[2]
Weight (kg)	**Females**	55.1 (9.9)	0.46 (2.74)	1.17 (2.96)	58.8 (10.9)	0.03 (3.11)	0.37 (2.11)	-0.36; [-2.68, 1.95]; 0.923	-0.61; [-3.09, 1.88]; 0.823
	Males	66.2 (9.6)	1.29 (3.25)	1.48 (3.09)	62.1 (8.27)	-0.82 (2.52)	-0.55 (3.86)	*-2.18; [-4.37, -0.01]; 0.051*	*-2.24; [-4.59, -0.11]; 0.065*
BMI (kg/m²)	**Females**	25.9 (5.32)	0.26 (1.3)	0.60 (1.45)	26.4 (4.67)	0.03 (1.4)	0.18 (0.97)	-0.23; [-1.21, 0.76]; 0.84	-0.40; [-1.43, 0.63]; 0.616
	Males	25.6 (3.41)	0.49 (1.22)	0.58 (1.14)	25.2 (3.88)	-0.37 (1.04)	-0.25 (1.49)	*-0.86; [-1.79, -0.07]; 0.074*	-0.84; [-1.815, -0.13]; 0.102
Bone mass (kg)	**Females**	2.17 (0.25)	0.03 (0.07)	0.02 (0.08)	2.25 (0.26)	-0.02 (0.08)	0.01 (0.08)	*-0.057; [-0.12, 0.00]; 0.064*	-0.01; [-0.074, 0.054]; 0.925
	Males	2.83 (0.26)	0.02 (0.08)	0.01 (0.09)	2.70 (0.24)	-0.03 (0.07)	-0.02 (0.09)	-0.046; [-0.10, 0.01]; 0.127	-0.04; [-0.1; 0.02]; 0.286
Body fat (kg)	**Females**	12.5 (5.73)	-0.24 (1.97)	0.70 (2.84)	14.6 (6.42)	0.19 (2.56)	0.16 (2.13)	0.55; [-1.12, 2.22]; 0.703	-0.46; [-2.44, 1.52]; 0.838
	Males	10.1 (5.28)	0.73 (2.34)	1.24 (2.32)	8.9 (5.54)	-0.35 (1.60)	-0.11 (2.51)	-1.152; [-2.72, -0.41]; 0.186	-1.39; [-3.24; 0.47]; 0.176

[1] Average treatment difference (EGCG vs. Placebo) after 12 months of treatment adjusted for baseline
[2] Average treatment difference (EGCG vs. Placebo) 6 months after treatment adjusted for baseline

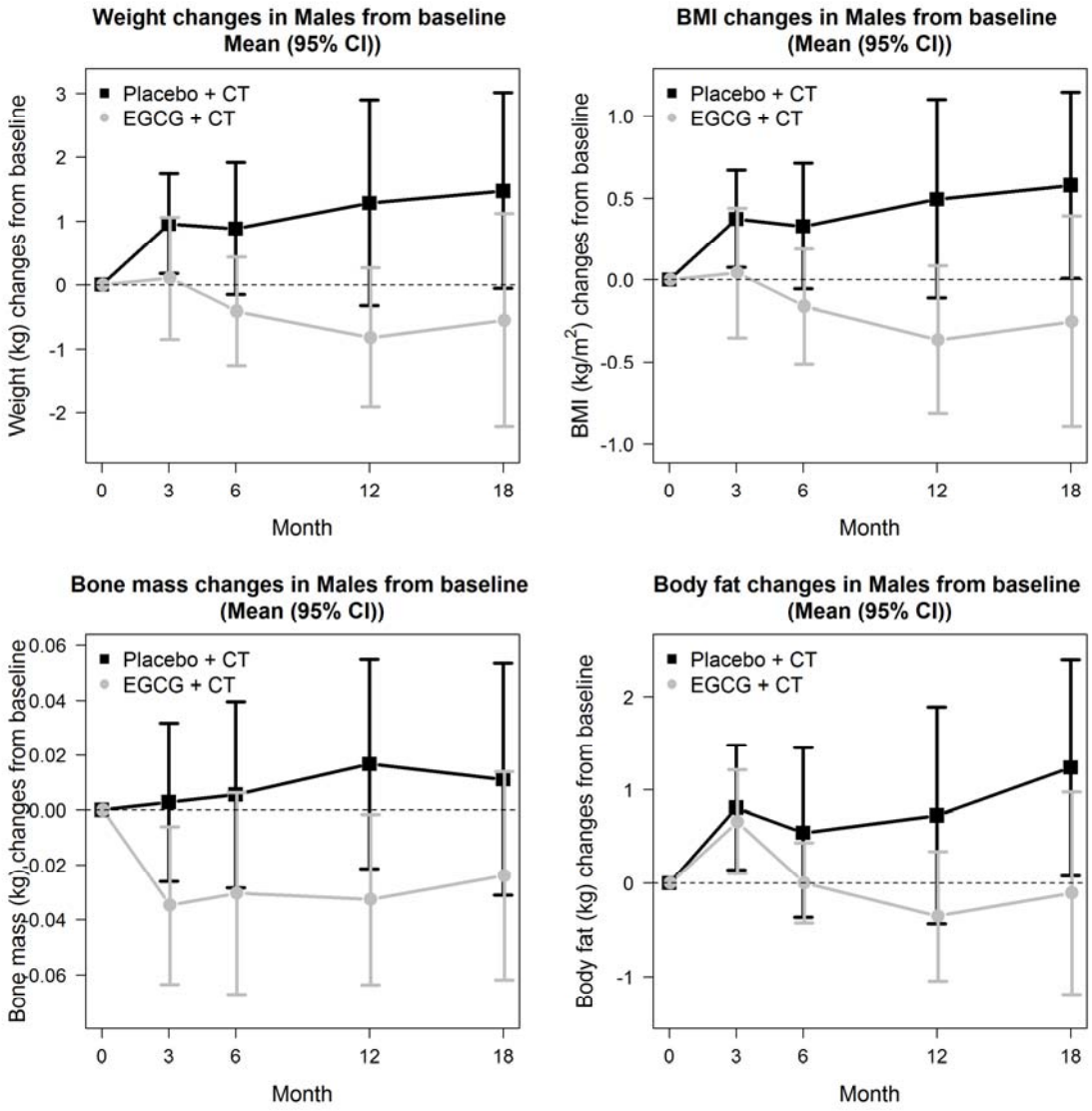

Figure 1: Changes in body composition in males from baseline to 12 months of intervention and 18 months (after 6 months of intervention discontinuation) (top to bottom: weight, BMI, bone mass, and body fat) for the two treatment groups (black: Placebo, grey: EGCG). Stars for significant differences between groups at 12 and 18 months: * $p<0.05$.

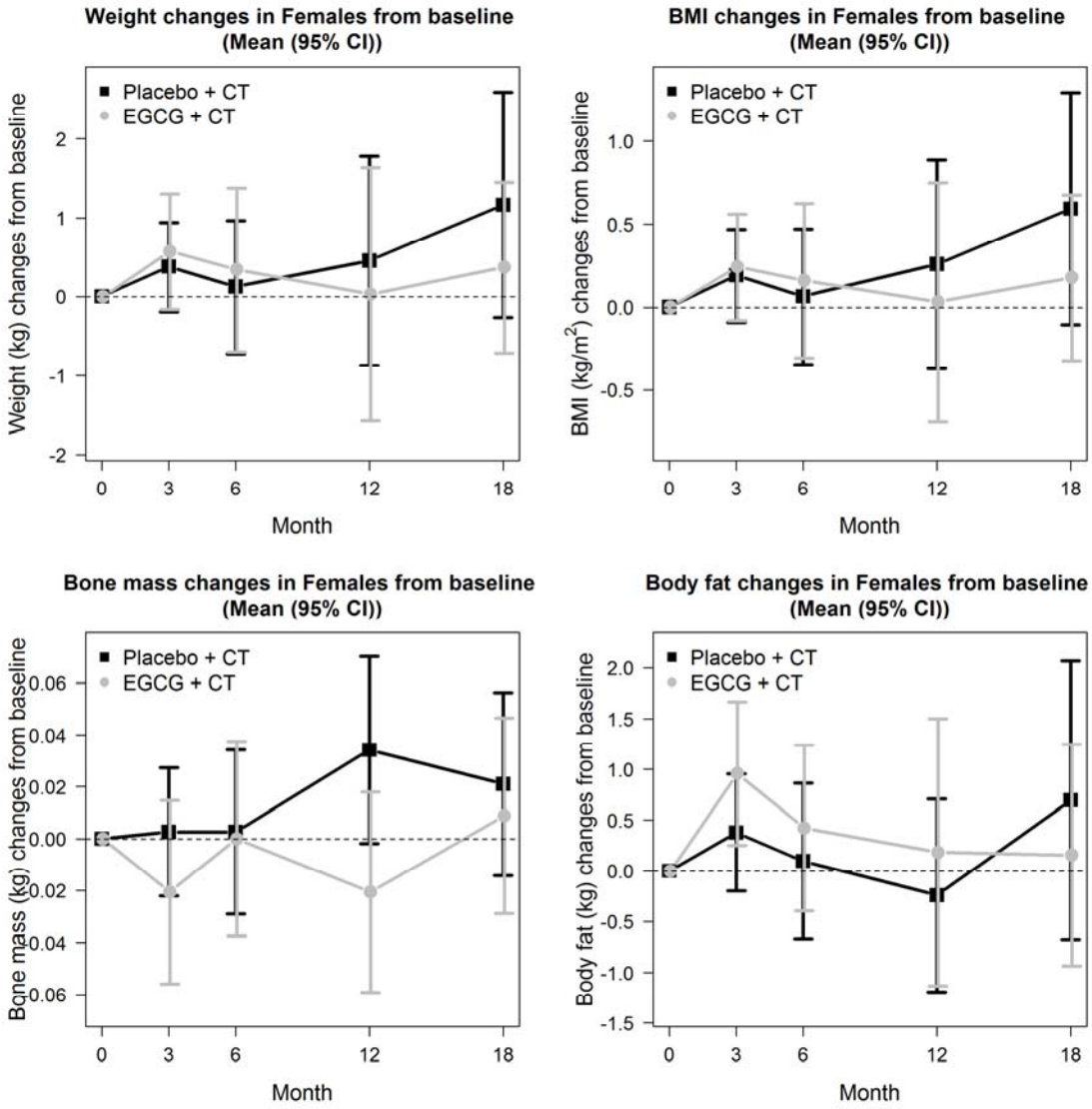

Figure 2: Changes in body composition in females from baseline to 12 months of intervention and 18 months (after 6 months of intervention discontinuation) (top to bottom: weight, BMI, bone mass, and body fat) for the two treatment groups (black: Placebo, grey: EGCG). Stars for significant differences between groups at 12 and 18 months: * $p<0.05$.

Changes in lipid profile

Following our results in body composition, we analyzed the changes in lipid profile at the same time points stratifying by sex. We did not observe a treatment effect in females neither after 12 months nor after 18 (**Table 3 and Figure 3**). In males, however, there were significant

differences at 12 months for TC and a trend for HDLc. At 18 months, no trends were observed (**Table 3 and Figure 4**).

Table 3: Baseline lipid profile values and differences from baseline after 12 and 18 months (mean; standard deviation) according to treatment and sex.

		Placebo + CT			EGCG + CT			AMD (95%-CI; p-value)	
		Baseline (Females n=19, Males n=18)	12 months (Females n=19, Males n=18)	18 months (Females n=19, Males n=18)	Baseline (Females n=17, Males n=23)	12 months (Females n=17, Males n=23)	18 months (Females n=17, Males n=23)	After 12 months[1]	After 18 months[2]
TC (mg/dL)	**Females**	176 (28.5)	-1.66 (19.7)	-3.71 (24.3)	174 (35.2)	-1.56 (16.7)	0.68 (23.8)	-0.33; [-13.43, 12.76]; 0.998	3.80; [-12.66, 20.26]; 0.842
	Males	165 (28.2)	8.89 (15.3)	10.22 (22.1)	178 (28.5)	-6.85 (18.2)	-4.80 (19.8)	**-12.9; [-25.42, -0.38]; 0.042**	-11.18; [-26.90, 4.55]; 0.209
Triglycerides (mg/dL)	**Females**	64.6 (23.8)	6.87 (24.0)	7.03 (27.0)	72.5 (27.1)	10.38 (34.1)	6.50 (20.4)	5.03; [-16.35, 26.40]; 0.84	2.95; [-19.59, 25.48.89]; 0.946
	Males	57.4 (24.9)	21.56 (27.9)	21.32 (22.1)	89.4 (38.9)	-3.67 (23.8)	5.15 (43.8)	-19.06; [-40.96, 2.85]; 0.1	-1.98; [-25.07, 21.12]; 0.976
HDLc (mg/dL)	**Females**	52.3 (11.3)	-3.69 (8.71)	-4.81 (6.84)	48.4 (9.28)	-4.29 (10.92)	0.37 (12.29)	-2.11; [-7.95, 3.74]; 0.66	3.42; [-2.45, 9.28]; 0.345
	Males	45.8 (7.3)	-0.34 (5.94)	-7.61 (7.48)	42.1 (5.44)	-4.30 (5.85)	-2.42 (5.83)	*-5.41; [-10.92, 0.10]; 0.055*	-3.36; [-8.89, 2.17]; 0.32
LDLc (mg/dL)	**Females**	111 (28.8)	0.66 (17.8)	-0.31 (18.1)	111 (31.3)	0.66 (13.2)	-0.99 (15.9)	0.04; [-11.26, 11.34]; 1	-0.66; [-14.54, 13.23]; 0.99
	Males	109 (26.3)	3.34 (10.6)	5.48 (17.8)	118 (24.0)	-1.82 (16.0)	-3.42 (18.9)	-3.65; [-14.56, 7.27]; 0.7	-7.64; [-21.08, 5.74]; 0.36
oxLDL	**Females**	44.2 (15.2)	-0.85 (12.3)	-3.66 (10.9)	45.4 (10.7)	-1.80 (13.27)	-2.95 (11.6)	-0.49; [-8.75, 7.76]; 0.99	-1.26; [-6.88, 9.38]; 0.93
	Males	46.1 (13.7)	-1.16 (10.4)	-0.97 (12.2)	51.5 (13.4)	-3.79 (9.78)	-5.51 (12.2)	-0.62; [-8.36, 7.12]; 0.98	-2.09; [-9.71, 5.53]; 0.78

[1] Average treatment difference (EGCG vs. Placebo) after 12 months of treatment adjusted for baseline

[2] Average treatment difference (EGCG vs. Placebo) 6 months after treatment adjusted for baseline

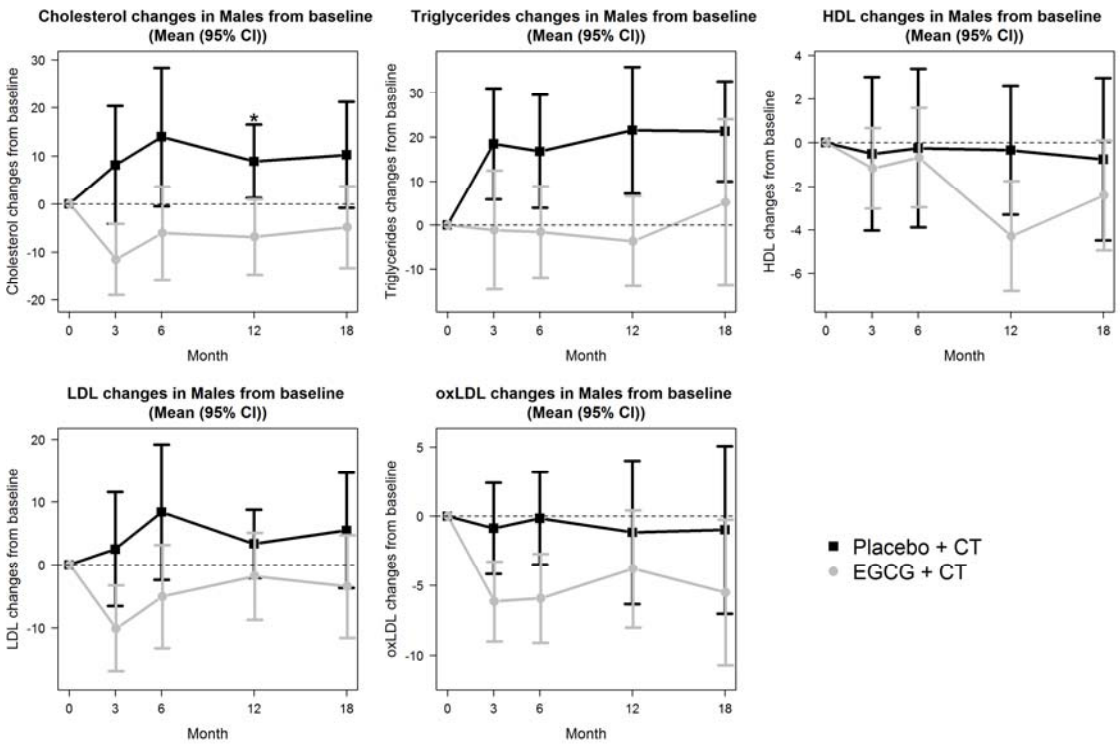

Figure 3: Changes in lipid profile in females from baseline (left to right and top to bottom: total cholesterol, triglycerides, HDLc, LDLc, and oxLDL) for the two treatment groups (black: Placebo, grey: EGCG). Stars for significant differences between groups at 12 and 18 months: * $p<0.05$.

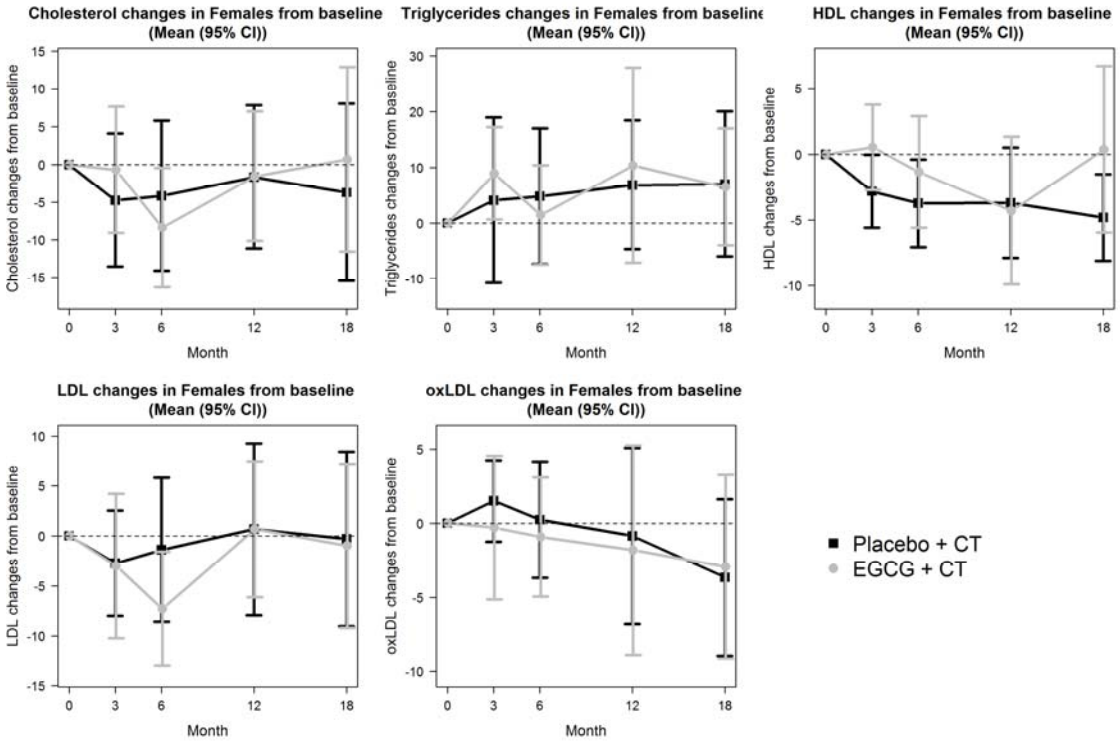

Figure 4: Changes in lipid profile in males from baseline (left to right and top to bottom: total cholesterol, triglycerides, HDLc, LDLc, and oxLDL) for the two treatment groups (black: Placebo, grey: EGCG). Stars for significant differences between groups at 12 and 18 months: * $p<0.05$.

Correlations between changes in body composition and lipid profile

Correlations between the differences from basal to 3, 6, 12, and 18 months were calculated for all variables related to body composition and lipid profile. We observed significant direct correlations between all the changes in body composition, except bone mass, and all those in lipid profile (Table 4), except for HDLc changes for which the only significant correlation was a direct one with the difference of percentage of the trunk fat. Due to the fact that individuals with DS have higher trunk fat, these correlations could mean that interventions lowering plasma lipids could be associated with lower trunk body fat.

Table 4: Correlations between differences from baseline for both body composition and lipid profile. Values are presented as Spearman's rho, 95% confidence interval, and p value.

	Cholesterol	Triglycerides	HDLc	LDLc	oxLDL
Weight	**0.239 [0.121,0.346] p< 0.001**	**0.185 [0.071,0.288] p=0.001**	0.059 [-0.053,0.169] p=0.3	**0.232 [0.121,0.339] p< 0.001**	**0.129 [0.009,0.246] p=0.012**
BMI	**0.232 [0.117,0.336] p< 0.001**	**0.184 [0.078,0.302] p=0.001**	0.053 [-0.067,0.174] p=0.35	**0.226 [0.111,0.327] p< 0.001**	**0.128 [0.006,0.238] p=0.013**
Body Fat	**0.298 [0.191,0.406] p< 0.001**	**0.300 [0.204,0.392] p< 0.001**	0.086 [-0.043,0.199] p=0.13	**0.261 [0.148,0.364] p< 0.001**	**0.218 [0.115,0.328] p< 0.001**
Body Fat percentage	**0.309 [0.204,0.412] p< 0.001**	**0.299 [0.194,0.394] p< 0.001**	*0.106 [-0.013,0.218]* p=*0.06*	**0.267 [0.162,0.370] p< 0.001**	**0.288 [0.124,0.333] p< 0.001**
% of trunk fat	**0.287 [0.178,0.400] p< 0.001**	**0.270 [0.155,0.375] p< 0.001**	**0.124 [0.009,0.243] p=0.03**	**0.243 [0.142,0.340] p< 0.001**	**0.232 [0.115,0.335] p< 0.001**
% of lower fat	**0.262 [0.161,0.368] p< 0.001**	**0.202 [0.161,0.368] p< 0.001**	0.043 [-0.081,0.158] p=0.46	**0.276 [0.160,0.376] p< 0.001**	**0.132 [0.019,0.238] p=0.01**
Bone mass	0.054 [-0.006,0.170] p=0.34	-0.058 [-0.169,0.048] p=0.31	-0.025 [-0.132,0.082] p=0.66	*0.096 [-0.018,0.200]* p=*0.09*	0.017 [-0.099,0.137] p=0.74

Discussion

In our study, young adult males with DS treated with EGCG showed a trend for less body weight gain (differential weight gain 2.18 kg, p=0.051) and lower BMI increase (0.86, p=0.074), than those receiving placebo along the treatment period. These effects were maintained after 6 months of treatment discontinuation. These results are similar to previous reports where decaffeinated green tea extract supplementation decreased body weight in men, and BMI after 8 weeks of green tea treatment [20], although in one study the effect was dependent on

247 seasonal weight fluctuations [21]. Regardless, this possible effect on weight management in DS
248 is relevant, due to the tendency of this population to have higher BMIs [3]

249 The observed reduction in fat gain may depend on the capability of green tea catechins to
250 decrease the absorption of fat and proteins, leading to reduced calorie intake [9]. EGCG has
251 also proven to ameliorate mitochondrial function and energy metabolism in DS [22, 23], which
252 could increase the metabolic rate leading to weight loss. Likewise, it is possible that EGCG has
253 an effect on postprandial glucose and satiety, as it has been observed for green tea [24], but
254 we lack the information on food consumption. However, we cannot ascertain whether these
255 mechanisms are behind the observed body composition changes. Other possible mechanisms
256 that could explain our results are related to the molecular actions of EGCG on AMPK which
257 increases energy metabolism, while decreasing gluconeogenesis in the liver (through inhibition
258 of PEPCK and G-6-Pase); or through a regulation of gene expression with an inhibition of genes
259 implicated in fat synthesis and an increase of lipolytic genes [8]. However, we cannot prove
260 that these particular mechanisms were involved in our study.

261 In the TESDAD study we observed a decrease in plasma lipid concentrations (including total
262 cholesterol) in the EGCG group that was significant at 12 months of treatment [12]. This effect
263 was subsequently found mainly in male participants, as shown by our stratified analyses. The
264 correlations found between the changes in body composition and lipid profile, indicate a
265 possible association, but more research is needed to ascertain the possible causative
266 relationship of the body weight on lipid profile. This lipid profile lowering effect has been
267 associated to EGCG-related decreased lipid absorption in rats [25], which could be linked to a
268 reduced gain in body weight.

269 In the case of the female participants, no significant EGCG effect on body composition was
270 observed. There were no differences in body weight, BMI, or any of the measures of body fat
271 versus the placebo condition. This lack of effect of EGCG on body composition in females

agrees with previous clinical studies in obese women [26]. However, further research into this sex-specific effect is needed, as other studies that included both sexes did not report it [27].

An unexpected outcome detected in the EGCG group, and particularly in females, was a lower bone mass in comparison with the placebo group. Although this effect was statistically significant, its clinical relevance is probably limited as it accounts for a difference of only 0.057 kg between treatments, which is lower than the criteria for pathological changes set at 2.5 SD [28], but we have no data on how this change in bone mass was distributed throughout the skeletal system. Additionally, the difference was only detected at 12 months of treatment, but disappeared after treatment discontinuation. This could parallel the negative effect on bone including structural and mechanical properties seen on both a Down syndrome mouse model and its wild-type littermates after a 50 mg/kg/day EGCG 7-week treatment [17]. Interestingly, a previous study of the same group observed beneficial effects of ~3mg/kg/day of EGCG on skeletal bone after a 3-week-treatment in Ts65Dn mice [16]. This suggests that the effect of EGCG on bone health is dose- and/or sex-dependent, and thus could not be observed in mice as all were male. As for which is the specific mechanism for the bone remodeling, a recent study analyzing the effect of EGCG on cranial morphology in children with DS hypothesized a role of NFAT through DYRK1A, which is inhibited by EGCG [18]. Whether the changes in bone mass could be considered an adverse event is discussable and needs further research. Most of the studies focusing on the adverse events related to green tea (including drinks with an equivalent dosage of 4-6 daily cups of green tea) and green extracts have focused on its possible hepatotoxicity. This adverse effect was considered to be rare even in the case of dry extracts and it has been sometimes considered to be linked to previous conditions (for a review see [29]). In our previous studies we deemed the administered dose of green tea extract to be safe [12, 19], with no observed alteration of liver function and with 124 adverse events reported (61 in placebo and 63 in EGCG group; mean: Placebo 1.79, EGCG 1.9), the most common adverse events were upper respiratory tract infection, osteoarticular pain,

mood disorders, menstrual disturbances, headache and mild abdominal pain. We observed some transient changes in the thyroid stimulating hormone (TSH). Increases in TSH concentrations were found to be more frequent in the treatment group (7 in treatment group versus 4 in placebo group). However, only one subject in the treatment group was diagnosed of hypothyroidism. Others were transient increases without concomitant T4 increase. Four subjects (2 in each group) had this value increased at 18 m visit, 6 months after discontinuation of the treatment. Therefore, the investigators considered this change not to be related to drug exposure.

Our study has several limitations given the lack of available data on metabolic and mitochondrial function, energy intake and expenditure, and satiety. Therefore, we cannot determine the specific mechanisms related to the effect on body composition elicited by EGCG, and thus further studies would need to collect additional data on diet, exercise, and metabolic state. Furthermore, we lack information on the menstrual cycle in the case of female participants, therefore we are unable to assess the effect of cyclical hormonal changes on our variables. Additionally, adolescents were included in the study without correcting for the Tanner scale score, despite its possible impact on both body composition and metabolism. The reason for this inclusion arises from the original clinical trial [12] were in order to avoid including individuals that could have dementia symptoms (i.e. older than 35), we included younger volunteers. Therefore, we acknowledge that this could be a confounding factor in our study, with those individuals who are 18 or under amounting for a total of the 11.7% of the sample. A further limitation is the fact that the sample size was determined based on the objective of the primary analysis, but not on the variable of the present study. For this reason, the statistical power of the tests applied may have been low.

In conclusion, EGCG modestly modulates body weight and composition in a normal weight adult population with DS. However, the clear sexual dimorphism we observed, with only males

benefiting from this effect, needs to be further explored. Additionally, there are correlations between the differences in both weight loss and body composition that need to be studied in order to analyze any possible causative relation. Of note the changes in bone mass detected in females should be taken into consideration when initiating treatments with EGCG or other catechins, being the changes in body composition monitored every 6 months.

Statement of Authorship

LX and JR contributed equally to the manuscript. JR collected the data; LX and KL performed the statistical analysis; MF performed lipids analysis; LX and RT wrote the manuscript. MD and RT conceived the idea, designed the trial, and supervised the work and the manuscript

Conflict of Interest Statement and Funding sources

No conflicts of interest are declared by coauthors.

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