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Resistance to lean mass gain in constitutional thinness in free-living conditions is not overpassed by overfeeding

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Abstract

Background Constitutional thinness (CT), a non-malnourished underweight state with no eating disorders, is characterized by weight gain resistance to high fat diet. Data issued from muscle biopsies suggested blunted anabolic mechanisms in free-living state. Weight and metabolic responses to protein caloric supplementation has not been yet explored in CT.

Methods A 2 week overfeeding (additional 600 kcal, 30 g protein, 72 g carbohydrate, and 21 g fat) was performed to compare two groups of CTs (12 women and 11 men) to normal-weight controls (12 women and 10 men). Bodyweight, food intake, energy expenditure, body composition, nitrogen balance, appetite hormones profiles, and urine metabolome were monitored before and after overfeeding.

Results Before overfeeding, positive energy gap was found in both CT genders (309 ± 370 kcal in CT-F and 332 ± 709 kcal in CT-M) associated with higher relative protein intake per kilo (1.74 ± 0.32 g/kg/day in CT-F vs. 1.16 ± 0.23 in C-F, $P < 0.0001$; 1.56 ± 0.36 in CT-M vs. 1.22 ± 0.32 in C-M, $P = 0.03$), lower nitrogen (7.26 ± 2.36 g/day in CT-F vs. 11.41 ± 3.64 in C-F, $P = 0.003$; 9.70 ± 3.85 in CT-M vs. 14.14 ± 4.19 in C-M, $P = 0.02$), but higher essential amino acids urinary excretion (CT/C fold change of 1.13 for leucine and 1.14 for arginine) in free-living conditions. After overfeeding, CTs presented an accentuated positive energy gap, still higher than in controls (675 ± 540 in CTs vs. 379 ± 427 in C, $P = 0.04$). Increase in lean mass was induced in both controls genders but not in CTs (a trend was noticed in CT women), despite a similar nitrogen balance after overfeeding (5.06 ± 4.33 g/day in CTs vs. 4.28 ± 3.15 in controls, $P = 0.49$). Higher anorectic gut hormones' tone, glucagon-like peptide 1 and peptide tyrosine tyrosine, during test meal and higher snacking frequency were noticed before and after overfeeding in CTs.

Conclusions The blunted muscle energy mechanism, previously described in CTs in free-living state, is associated with basal saturated protein turn over suggested by the concordance of positive nitrogen balance and an increased urine excretion of several essential amino acids. This saturation cannot be overpassed by increasing this spontaneous high-protein intake suggesting a resistance to lean mass gain in CT phenotype.

Keywords Overfeeding; Constitutional Thinness; Bodyweight gain; Energy gap; Nitrogen balance

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Background

Bodyweight maintenance is related to the balance between energy intake and expenditure, with interindividual physiological variations, from thinness to obesity.[1] A recent cohort publication showed the difference of naturally underweight women from anorexia nervosa.[2] Indeed, this low bodyweight condition [body mass index (BMI) $<$ 17.5 kg/m^2] also called constitutional thinness (CT) is a state without any sign of undernutrition as observed through normal nutritional biomarkers and gonadal function and low but not blunted leptin plasma levels.[1–5] CT also present with less psychological dietary restriction behaviours compared with normal-weight women.[2] CT individuals have a steady bodyweight in the lower population-wide percentiles adjusted for age, gender, and ethnicity,[2,3] suggestive of a genetic determination of this underweight state consistent with previous reports on the heritability of thinness.[6–11] The CT condition also impacts one's quality of life, CT patients being generally unsatisfied by their low body weight and inability to gain weight and often consult for medical advice.[2,12]

A more mechanistic understanding of this phenotype is currently lacking yet would be important to identify potential therapeutic targets. As a proof of concept, a recent 4 week fat overfeeding study, an excess of more than 600 kcal per day in fat, revealed a form of resistance to bodyweight and fat mass gain in a group of CT women, and that despite an enhanced paradoxical positive energy gap (higher food consumed than energy expended).[13] This could reflect the paradoxical negative energy gap suggested in genetic obesity.[14] Although this gap may be interpreted as a food intake reporting issue, hypothesis in energy pathways specific to CT population should not be neglected. Recently, we have shown the CT condition is also marked by a distinct skeletal muscle phenotype, as energy storage defects were observed in muscle biopsies that may partly contribute to body weight gain resistance.[15] Yet molecular processes behind protein storage and turn over are poorly understood in this population.

In the present study, we aimed at further studying the protein metabolism of CT male and female subjects compared with control individuals. We conducted an overfeeding intervention in both groups using Renutryl® Booster to provide additional 600 kcal, 30 g protein, 72 g carbohydrate, and 21 g fat intake per day for 2 weeks. Using a combination of anthropometric, clinical, and metabolic phenotyping measures, we explored clinical end points related to energy and protein balance, appetite regulation, and 24 h urinary metabolomics.[16]

Methods

Ethics

This clinical investigation was conducted in accordance with the ethical standards laid down in the 1964 Declaration of Helsinki and its later amendments (as revised in 1983). Study was approved by the local research and ethics committee of Saint-Etienne, France, ANSM (2013-A00590-45) and registered at clinicaltrial.gov as NCT02004821. All subjects gave a written informed consent.

Subjects

A total of 67 healthy young subjects were recruited, of which a total of 60 completed the study protocol (dropouts: one female CT, three male CTs, two female controls, one male control): 15 female CT (CT-F), 15 male CT (CT-M), 15 female controls (C-F), and 15 male controls (C-M) (see Supporting information, **Supplementary figure 1**).

CT subjects (BMI $<$ 18.5 kg/m^2) were recruited amongst our outpatients all of whom wished to gain weight, had stable bodyweight throughout post-pubertal period confirmed by their personal weight history, without eating disorders as evaluated by the Eating Disorder Examination Questionnaire and Dutch Eating Behavior Questionnaire, and no amenorrhoea for women after hormonal contraception withdrawal when relevant.[2] CT non-undernourished state of underweight was supported by normal nutritional biomarkers including normal levels of insulin-like growth factor 1 (IGF-1) and free triiodothyronine (FT_3).[2] They displayed no hepatic disorders and no over exercise behaviour according to the MONICA Optional Study of Physical Activity Questionnaire.[17]

Normal-weight subjects (BMI 20–25 kg/m^2) without any eating disorders and any medication were recruited by advertising to serve as control group.

All subjects were recruited within an age range between 18 and 36 years, had a stable body weight for at least 3 months, and did not take any medication. All criteria of inclusion and exclusion were previously fully detailed.[16]

Study design and dietary intervention

After 2 week of baseline assessments (day D1–D11, visits V1–V4), all participants were asked to consume an extra bottle of Renutryl® Booster [72 g carbohydrates (48.5%), 30 g proteins (20%), and 21 g fat (31.5%)] as an add-on to their usual food intake during 14 consecutive days (D11–D25, V5–V8).

Participants were required to consume the bottle in the interval of dinner-to-bed time in free-living conditions and to maintain their usual dietary and normal lifestyle throughout the study. Bodyweight was measured throughout the study and was followed-up at 2 week post-overfeeding (ad libitum free-living period) (D43, V9). Scheduled appointments allowed to regularly check the compliance in order to avoid compensatory behaviours.[18] Study protocol design was previously extensively described in a specific design article.[16]

Anthropometric and body composition measurements

All subjects underwent anthropometric measurements in the early morning at fasting state at each visit. Total body weight (TBW) was measured with 0.1 kg precision and height with 0.1 cm. Body composition was assessed, at baseline and at post-overfeeding, to determine the distribution of total body fat mass (FM) and fat-free mass using dual-energy X-ray absorptiometry.[19]

Energy balance assessments

Energy balance assessments were performed before and after overfeeding both under free-living and experimental conditions.

Under free-living conditions

Resting energy expenditure (REE) was measured at 12 h fasting state in supine position by indirect calorimetry using a canopy (Quark RMR, COSMED, Italy).[20] Accelerometer (Actiheart, CamNtech, Cambridge, UK)[21] was used to monitor physical activity level (PAL) in real life. Free-living total energy expenditure (TEE) was obtained from calculation: TEE = REE × PAL. Fat and carbohydrate oxidation was assessed using Ferrannini's equations.[22]

Usual caloric consumption and eating habits were assessed using a daily self-reporting dietary record during 7 days, with the guide of a photographic reference book previously validated.[23]

Dietary record was checked by a dietician to ensure accuracy of data collection. Total daily energy intake (TEI) was calculated using GENI software (MICRO6, France) and the French Food and Nutrient Files CNEVA-CIQUAL.[24] Snacking was defined as food intake out of the 3 main meals (breakfast, lunch and dinner). The ratio of TEI/REE was used to detect misreporting of dietary intake, with underreporting <1.35 and overreporting >2.10.[25]

Under experimental conditions

Experimental TEE was measured using a whole-body calorimetric chamber, an open-circuit indirect calorimetric system (HNRC, Auvergne, France), previously described.[26] Volunteers spent 38 h in calorimetric chambers. They entered at 5 p.m. the first evening for an adaption night, and the experiment started at 7 a.m. the next time, and ended 24 h later. Identical standardized controlled meals consisting of 2300 kcals per day (three main and one snacking in the afternoon) were given to participants, enough to cover their usual food intake according to ambulatory evaluation. No extra outside food was allowed. Real food intake was measured by dietitians in order to be accurate with simultaneous energy expenditure measurements.

Samplings

Baseline venous samples were collected after a 12 h overnight fasting for the measurement of serum leptin, albumin, free T3, IGF-1, insulin, blood glucose, triglycerides, non-esterified fatty acids, and glycerol. Homeostatic Model assessment of insulin resistance was calculated according to the formula: fasting insulin (μUI/L) × fasting glucose (mmol/L)/22.5.[27]

Twenty-four hour urine samples were collected for nitrogen loss determination as well as metabolome analysis at different visits, including baseline visits (V2 and V3) and visits during overfeeding (V6, V7, and V8).

Standardized test meals were performed before (V2), and at the end of the 2 week overfeeding period (V8), using a bottle of Renutryl® Booster, consumed slowly during 15 min under surveillance. Venous blood samples were collected in tubes containing aprotinin and EDTA at 7 time points: T_0 = 0 min (after a 12 h overnight fasting), T_{15} = 15 min, (immediately after Renutryl® Booster consumption), T_{30} = 30 min, T_{60} = 60 min, T_{90} = 90 min, T_{120} = 120 min, and T_{150} = 150 min, to assay blood glucose, insulin, total ghrelin and acyl-ghrelin (AG), peptide tyrosine tyrosine (PYY), and glucagon-like peptide 1 (GLP-1) concentrations. Samples were immediately centrifuged at +4°C, aliquoted, and kept frozen at −20°C before stocked at −80°C until assays. A 1 N HCl was added to the final concentration of 0.1 HCl into an aliquot dedicated to ghrelin assay in order to enhance acylated ghrelin stability.

Assays

Standardized techniques for assessment of plasma parameters were previously described.[16] Urinary urea/24 h was measured based on enzymatic reaction with urease and glutamate dehydrogenase. Nitrogen (N) intake was assessed with self-reported diet diaries (free living) and controlled weighed food by dietician (calorimetric chamber), with 6.25 g of protein per g of N. N losses were calculated based on Lee and Hartley formula: nitrogen excretion (g N/24 h) = urinary urea (mmol/24 h) × 0.028 × 1.2 (factor of non-urea urine N losses).

Urinary metabolomics

Following homogenization, aliquots of 24 h urine samples were prepared and kept frozen at −80°C until analysis. Metabolomics analysis was carried out by ^{1}H NMR spectroscopy in-house at NIHS, Switzerland, using established procedures.[28] Exhaustive description of the method is presented in the supplementary method. A total of 79 signals out of 155 were assigned to biochemical molecular species, corresponding to 51 unique metabolites. The signals were expressed in arbitrary units corresponding to peak area normalized to total spectral area or creatinine peak area. This approach allowed for the detection of major metabolic intermediates belonging to central metabolism, including amino acids, organic acids, and sugars, as well as aromatic-containing compounds.

Statistical analysis

In the current study, only participants with complete data and who really complied with the overfeeding were included in statistical analyses. Compliance was defined using the following criteria: increased food intake above 450 kcal per day,[13] positive change in urine urea,[29] and no increase in PAL during the overfeeding period. Intergroup differences and the effects of the short-term overfeeding within each group were evaluated in 12 female CTs (CT-F), 11 male CTs (CT-M), 12 female controls (C-F), and 10 male controls (C-M).

All data are presented as mean ± SD. Homogeneity of data was checked with the Kolmogorov–Smirnov test, and transformation was applied when data were not normally distributed.

Mann–Whitney's non-parametric unpaired test was used to compare one-time measured parameters (including meal test mean values) between CT and controls groups (including both genders and for each gender separately) at each visit. Wilcoxon signed rank's non-parametric tests were used to analyse the differences before vs. after overfeeding points for a given parameter within each group (including both genders and for each gender separately). Correlations between every baseline parameters and basal gap were also evaluated in order to find out potential predictive markers of the energy gap. Statistical significance was set at $P < 0.05$.

A one-factor (time) repeated measures analysis of variance was used to analyse the parameters changes over the visits and appetite-regulatory hormones changes during each test meal in each group (including both genders and for each gender separately). Fisher's PLSD post hoc tests between two assessment points were performed when time effect was significant ($P < 0.05$).

Multiple testing was taken into account by correcting the *P* values using the Bonferroni method.

Statistical analyses and graphs were performed with StatView 4.5 (Abacus Concepts, Inc. CA) and GraphPad Prism 5.0 software (GraphPad Software, San Diego, CA).

Regarding the urinary metabolomics data, initial data analyses were conducted using principal component analysis in order to assess metabolic similarities between samples and outlier detection. Further characterization of discriminating metabolite phenotype was performed using supervised multivariate data analysis of total area and creatinine normalized data, using orthogonal projection to latent structures discriminant analysis (orthogonal partial least squares discriminant analysis,[30]). To analyse metabolites longitudinal information, we applied an analysis of variance for repeated measurements with age and sex as confounders and CT status as main effect. An interaction term between visit and status parameters was added to the model to assess a potential different metabolite trajectory between the two groups. All metabolites were log2 transformed before analysis. The same analysis was also performed after a rank transformation to normality of the metabolites values to check for false positive results because of an excess of outliers or an excess of distribution skewness. Correction for multiple testing was applied using the Benjamini–Hochberg method.

Results

Baseline characteristics

Constitutional thinness subjects in both genders had lower mean body weight, BMI, fat mass (absolute and relative values), lean mass (absolute value), and leptin levels, as compared with control subjects. However, there were no differences between CTs and controls in nutritional markers, including albumin, triglycerides, non-esterified fatty acids, and glycerol, free T_3, and IGF-1. All subjects were insulin-sensitive displaying normal glucose tolerance (*Table* 1).

Free-living TEI in kcal was slightly higher in female CTs compared with female controls and was similar between CTs and male controls. Daily snacking calorie contribution was significantly greater in CTs compared with controls (mean 16.76 ± 10.31% vs. 10.55 ± 8.65%, respectively, $P = 0.0142$). Baseline food-reporting mean ratio was calculated at 1.325 ± 0.052 in female controls; 1.937 ± 0.113 in female CTs; 1.482 ± 0.057 in male controls, and 1.684 ± 0.092 in male CTs (*Table* 2).

Absolute protein intake was similar between CTs and controls in both genders (*Table* 3). However, when reported to bodyweight as proposed in protein dietary reference intakes, CTs' protein intake was significantly higher compared with controls in both genders (*Table* 3). Absolute nitrogen losses were significant lower in both female and male CTs, and

Table 1 Baseline clinical characteristics of all participants

Parameters	CT-F	C-F	*P* value (females)	CT-M	C-M	*P* value (males)
Body composition						
Age (years)	26.9 ± 4.7	21.9 ± 3.0	0.0018	23.0 ± 3.9	23.3 ± 2.8	0.7889
BMI (kg/m^2)	16.57 ± 0.72	22.88 ± 1.17	<0.0001	17.31 ± 0.73	22.94 ± 0.99	<0.0001
Weight (kg)	42.85 ± 4.44	62.26 ± 4.72	<0.0001	53.57 ± 2.73	74.86 ± 7.08	<0.0001
Nutritional biomarkers						
Leptin (ng/mL)	4.53 ± 1.85	13.29 ± 6.22	<0.0001	0.95 ± 0.09	3.30 ± 2.80	0.0031
Free T3 (pmol/L)	6.2 ± 2.7	5.4 ± 0.6	0.2999	5.6 ± 0.6	5.3 ± 0.8	0.3970
IGF-1 (μg/L)	239 ± 17	274 ± 10	0.0893	221 ± 29	255 ± 21	0.3938
Albumine (g/L)	0.26 ± 0.06	0.28 ± 0.04	0.5408	0.30 ± 0.04	0.30 ± 0.05	0.9906
Metabolic parameters						
Fasting insulin (UI/L)	5.68 ± 2.33	7.31 ± 2.18	0.0629	6.43 ± 4.61	7.41 ± 3.39	0.5122
Fasting blood glucose (nmol/L)	4.61 ± 0.41	4.61 ± 0.50	0.9999	4.67 ± 0.28	4.71 ± 0.38	0.7844
HOMA-IR	1.18 ± 0.53	1.51 ± 0.53	0.1061	1.35 ± 1.00	1.57 ± 0.75	0.5008
TG (mmol/L)	0.96 ± 0.27	0.82 ± 0.33	0.2348	0.90 ± 0.25	0.91 ± 0.25	0.8699
NEFA (μmol/L)	459 ± 236	477 ± 239	0.8335	313 ± 126	251.6 ± 140	0.2111
Glycerol (μmol/L)	45.4 ± 31.4	34.1 ± 22.1	0.2874	15.8 ± 13.8	14.1 ± 11.8	0.7148

Mean ± SD. Statistical significance when *P* value <0.05.
BMI, body mass index; C, controls; CT, constitutional thinness; F, female; HOMA-IR, homeostatic model assessment for insulin resistance; NEFA, non-esterified fatty acids; M, male; TG, triglycerides.

Table 2 Energetic and metabolic parameters for all groups at baseline and at post-overfeeding

		Baseline			Post-overfeeding				
Parameters		CTs	Controls	*P* value	CTs	Controls	*P* value	Time *P* value (CTs)	Time *P* value (controls)
Fat mass (kg)	Female	10.3 ± 1.5	19.7 ± 2.9	<0.0001	10.5 ± 1.3	19.9 ± 2.7	<0.0001	0.1485	0.0500
	Male	8.2 ± 1.2	17.1 ± 6.8	<0.0001	8.6 ± 1.5	17.3 ± 6.7	<0.0001	0.0183	0.0566
	Total	9.3 ± 1.7	18.5 ± 5.1	<0.0001	9.6 ± 1.7	18.7 ± 5.0	<0.0001	0.0051	0.0050
Lean mass (kg)	Female	32.4 ± 2.7	42.4 ± 3.2	<0.0001	32.9 ± 3.1	43.3 ± 2.9	<0.0001	0.0733	0.0011
	Male	45.4 ± 2.5	58.1 ± 6.5	<0.0001	45.6 ± 2.2	59.0 ± 6.9	<0.0001	0.4425	0.0067
	Total	38.9 ± 7.1	49.5 ± 9.4	<0.0001	39.3 ± 7.0	50.4 ± 9.4	<0.0001	0.0665	<0.0001
Resting energy	Female	1059 ± 129	1344 ± 103	0.0007	1119 ± 122	1377 ± 124	<0.0001	0.0021	0.3325
expenditure	Male	1444 ± 130	1590 ± 280	0.0005	1398 ± 191	1762 ± 238	<0.0001	0.7588	0.0090
(kcal/24 h)	Total	1252 ± 233	1456 ± 234	<0.0001	1258 ± 212	1552 ± 266	<0.0001	0.1302	0.0075
Free living	Female	2039 ± 306	1788 ± 303	0.0144	2553 ± 254	2429 ± 240	0.2306	0.0002	<0.0001
Total energy	Male	2425 ± 534	2349 ± 583	0.9971	2618 ± 430	2986 ± 514	0.1035	0.1459	0.0010
intake	Total	2232 ± 469	2043 ± 525	0.2043	2586 ± 386	2681 ± 473	0.4526	0.0003	<0.0001
(kcal/24 h)									
Free living	Female	1730 ± 266*	1989 ± 325*	0.0439	1792 ± 275*	2011 ± 328*	0.0901	0.2794	0.8168
Total energy	Male	2093 ± 429*	2506 ± 607	0.0935	2029 ± 539*	2651 ± 588*	0.0176	0.6298	0.0949
expenditure	Total	1912 ± 429*	2224 ± 532	0.0330	1911 ± 436*	2302 ± 558*	0.0107	0.7520	0.1048
(kcal/24 h)									
Calorimetric	Female	1903 ± 215	2022 ± 169	0.1464	2464 ± 225	2635 ± 191	0.0563	<0.0001	<0.0001
chamber	Male	2082 ± 173	2166 ± 152	0.2461	2583 ± 290	2738 ± 155	0.1461	<0.0001	<0.0001
Total energy	Total	1993 ± 212	2088 ± 174	0.1056	2523 ± 261	2682 ± 179	0.0217	<0.0001	<0.0001
intake									
(kcal/24 h)									
Calorimetric	Female	1616 ± 186*	2003 ± 187	<0.0001	1607 ± 2011*	2141 ± 167*	<0.0001	0.9754	0.0004
chamber	Male	1998 ± 138	2398 ± 252	<0.0001	2063 ± 146*	2498 ± 206*	<0.0001	0.0666	0.0167
Total energy	Total	1807 ± 252*	2182 ± 293	<0.0001	1835 ± 293*	2303 ± 257*	<0.0001	0.2643	<0.0001
expenditure									
(kcal/24 h)									
Fat oxidation	Female	1.2 ± 0.4	1.3 ± 0.4	0.7031	0.8 ± 0.4	0.8 ± 0.5	0.7415	0.0444	0.0128
rate	Male	0.9 ± 0.4	0.9 ± 0.4	0.4857	0.9 ± 0.3	0.7 ± 0.4	0.0731	0.6693	0.3012
(mg/min/kg)	Total	1.1 ± 0.4	1.1 ± 0.4	0.9776	0.9 ± 0.3	0.8 ± 0.3	0.3985	0.0794	0.0081
Carbohydrate	Female	1.5 ± 0.5	0.9 ± 0.6	0.0139	2.2 ± 1.1	1.9 ± 0.9	0.4605	0.0332	0.0167
oxidation rate	Male	2.1 ± 1.2	1.3 ± 0.7	0.0812	1.7 ± 0.8	2.2 ± 0.8	0.1685	0.4158	0.0288
(mg/min/kg)	Total	1.8 ± 0.9	1.1 ± 0.7	0.0063	1.9 ± 0.9	2.0 ± 0.9	0.7586	0.5743	0.0008

Data are expressed as mean ± SD.
CT, constitutional thinness.
*$P < 0.05$ between TEI and TEE (gap) for each group in each condition at each time of the study.

Table 3 Nitrogen balance assessed in two conditions: in free living and in calorimetric chamber

			Baseline			Post-overfeeding				
	Parameters		CTs	Controls	*P* value	CTs	Controls	*P* value	Time *P* value (CTs)	Time *P* value (controls)
Free living	Mean carbohydrates intake (g)	Female	238.0 ± 42.1	201.1 ± 32.5	0.0250	305.1 ± 44.6	279.3 ± 38.3	0.1435	0.0001	<0.0001
		Male	284.6 ± 80.7	269.0 ± 73.7	0.6595	308.0 ± 36.0	351.6 ± 78.9	0.1141	0.0034	0.2952
		Total	260.3 ± 66.4	230.2 ± 62.7	0.1306	306.5 ± 39.8	312.2 ± 69.2	0.7350	0.0012	<0.0001
	Mean fat intake (g)	Female	84.4 ± 14.5	73.8 ± 18.7	0.1328	99.8 ± 9.7	96.9 ± 12.4	0.5377	0.0068	<0.0001
		Male	91.9 ± 21.8	84.3 ± 19.5	0.4116	95.8 ± 18.2	113.1 ± 21.1	0.0585	0.5428	0.0038
		Total	88.0 ± 18.3	78.5 ± 19.3	0.0997	97.9 ± 14.2	104.3 ± 18.4	0.1972	0.0197	<0.0001
	Mean protein intake (g/day)	Female	72.67 ± 13.24	71.33 ± 14.01	0.8129	101.50 ± 7.94	100.67 ± 6.50	0.7810	<0.0001	<0.0001
		Male	83.55 ± 20.41	90.80 ± 22.30	0.4459	102.55 ± 18.23	124.50 ± 17.96	0.0120	0.0060	<0.0001
		Total	77.87 ± 17.55	80.18 ± 20.35	0.6848	102.00 ± 13.52	111.50 ± 17.55	0.0476	<0.0001	<0.0001
	Mean protein intake per weight (g/kg/day)	Female	1.74 ± 0.32	1.16 ± 0.23	<0.0001	2.35 ± 0.18	1.60 ± 0.16	<0.0001	<0.0001	<0.0001
		Male	1.56 ± 0.36	1.22 ± 0.32	0.0345	1.89 ± 0.37	1.64 ± 0.30	0.1116	0.0106	<0.0001
		Total	1.65 ± 0.34	1.18 ± 0.27	<0.0001	2.13 ± 0.37	1.62 ± 0.22	<0.0001	<0.0001	<0.0001
	N intake (g/day)	Female	11.63 ± 2.12	11.41 ± 2.24	0.8129	16.24 ± 1.27	16.11 ± 1.04	0.7810	<0.0001	<0.0001
		Male	13.37 ± 3.27	12.73 ± 3.57	0.4459	16.41 ± 2.92	19.92 ± 2.87	0.0120	0.0060	<0.0001
		Total	12.46 ± 2.81	12.83 ± 3.26	0.6848	16.32 ± 2.16	17.84 ± 2.81	0.0476	<0.0001	<0.0001
	N losses (g/day)	Female	7.26 ± 2.38	11.41 ± 3.64	0.0032	10.90 ± 3.69	12.73 ± 2.30	0.1598	0.0243	0.0936
		Male	9.70 ± 3.85	14.14 ± 4.19	0.0202	11.66 ± 4.79	14.56 ± 3.10	0.1208	0.1645	0.8291
		Total	8.43 ± 3.33	12.65 ± 4.05	0.0004	11.27 ± 4.17	13.56 ± 2.79	0.0364	0.0070	0.3339
	N balance (g/day)	Female	4.37 ± 3.11	0 ± 2.84	0.0016	5.34 ± 3.95	3.38 ± 2.20	0.1470	0.5098	0.0010
		Male	3.67 ± 4.07	0.39 ± 3.30	0.0582	4.75 ± 4.88	5.36 ± 3.85	0.7532	0.5167	0.0089
		Total	4.03 ± 3.53	0.18 ± 2.99	0.0003	5.06 ± 4.33	4.28 ± 3.15	0.4969	0.3380	<0.0001
Calorimetric chamber	Mean protein intake (g/day)	Female	67.37 ± 7.50	73.12 ± 7.82	0.0797	95.44 ± 8.97	104.68 ± 6.53	0.0086	<0.0001	<0.0001
		Male	71.33 ± 8.99	76.79 ± 7.10	0.1420	97.12 ± 10.54	106.41 ± 7.39	0.0311	<0.0001	<0.0001
		Total	69.27 ± 8.31	74.79 ± 7.56	0.0246	96.24 ± 9.52	105.47 ± 6.82	0.0006	<0.0001	<0.0001
	Mean protein intake per weight (g/kg/day)	Female	1.61 ± 0.22	1.18 ± 0.11	<0.0001	2.23 ± 0.21	1.66 ± 0.10	<0.0001	<0.0001	<0.0001
		Male	1.34 ± 0.20	1.02 ± 0.14	0.0007	1.78 ± 0.22	1.39 ± 0.16	0.0002	<0.0001	<0.0001
		Total	1.48 ± 0.25	1.11 ± 0.14	<0.0001	2.01 ± 0.31	1.54 ± 0.19	<0.0001	<0.0001	<0.0001
	N intake (g N/day)	Female	10.78 ± 1.20	11.67 ± 1.25	0.0797	15.27 ± 1.44	16.75 ± 1.05	0.0086	<0.0001	<0.0001
		Male	11.41 ± 1.44	12.29 ± 1.14	0.1420	15.54 ± 1.67	17.03 ± 1.18	0.0311	<0.0001	<0.0001
		Total	11.08 ± 1.33	11.97 ± 1.21	0.0246	15.40 ± 1.52	16.88 ± 1.09	0.0006	<0.0001	<0.0001
	N losses (g N/day)	Female	8.74 ± 1.35	9.81 ± 1.79	0.1135	12.14 ± 3.24	13.26 ± 1.59	0.2924	0.0044	<0.0001
		Male	10.33 ± 1.60	12.98 ± 2.54	0.0093	13.25 ± 2.12	16.18 ± 2.65	0.0111	<0.0001	0.0002
		Total	9.50 ± 1.66	11.25 ± 2.66	0.0108	12.67 ± 2.76	14.59 ± 2.55	0.0200	<0.0001	<0.0001
	N balance (g N/day)	Female	2.04 ± 0.91	1.89 ± 1.92	0.8124	3.13 ± 3.00	3.49 ± 1.84	0.7266	0.1287	0.0052
		Male	1.08 ± 1.61	−0.70 ± 2.46	0.0624	2.29 ± 1.90	0.85 ± 2.48	0.1486	0.0031	0.0152
		Total	1.58 ± 1.35	0.71 ± 2.50	0.1531	2.73 ± 2.49	2.29 ± 2.49	0.5552	0.0052	0.0001

N intake was assessed with self-reported diet diaries (free living) and controlled weighed food by dietician (chamber), with 6.25 g of protein per grams of N. N losses was calculated based on Lee and Hartley formula: nitrogen excretion (g N/24 h) = urinary urea (mmol/24 h) × 0.028 × 1.2 (factor of non-urea urine N losses). A 30 g of protein provided by Renutryl gives 4.8 g of N on top of daily N intake during the overfeeding period.
CT, constitutional thinness; N, nitrogen.

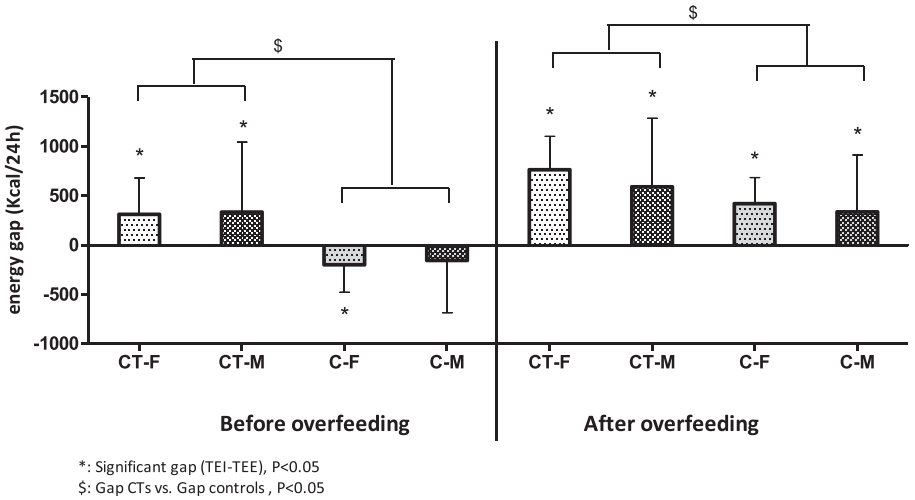

Figure 1 Energy gap in free living in all groups before and after overfeeding. Data are expressed as mean ± SD. *significant gap (TEI–TEE), $P < 0.05$ and $gap CTs vs. gap controls, $P < 0.05$.

nitrogen balance was found positive and significantly increased in CTs compared to their counterparts (*Table* 3).

Baseline free-living REE was significantly lower in CTs than in controls in both genders (*Table* 2). Once adjusted to LM, REE/LM was significantly greater in CTs men only as compared with their controls (31.7 ± 3.1 kcals/kg vs. 28.0 ± 3.3 kcals/kg, $P = 0.004$). TEE, assessed in both conditions (free living and chamber), was significantly lower in CTs compared to controls in both genders (*Table* 2).

Fasting fat oxidation was similar between CTs and controls, whereas fasting carbohydrate oxidation was higher in CTs women than in their controls (*Table* 2).

A positive energy gap was found in CTs in free-living conditions, in both genders. Controls tended to present a negative gap (*Table* 2 and *Figure* 1). In experimental condition (i.e. 24 h stay in the calorimetric chamber), we found a positive gap only in female CTs and a negative one in male controls (*Table* 2).

Correlation analysis between energy gap and baseline parameters in free-living showed strong correlation with snack calorie intake ($P = 0.0002$, $R^2 = 0.22$) and relative protein intake expressed per bodyweight (kg) ($P < 0.0001$, $R^2 = 0.512$).

Energy and metabolic response to overfeeding (Table 2)

The short-term protein-energy overfeeding paradigm induced a bodyweight gain in all groups. The gained weight was maintained after 2 week post-overfeeding only in women (*Figure* 2). Overall calculated total bodyweight gain (TBW_{V8}–TBW_{V2}) trend to be higher in controls than in CT (1.16 ± 0.15 vs.

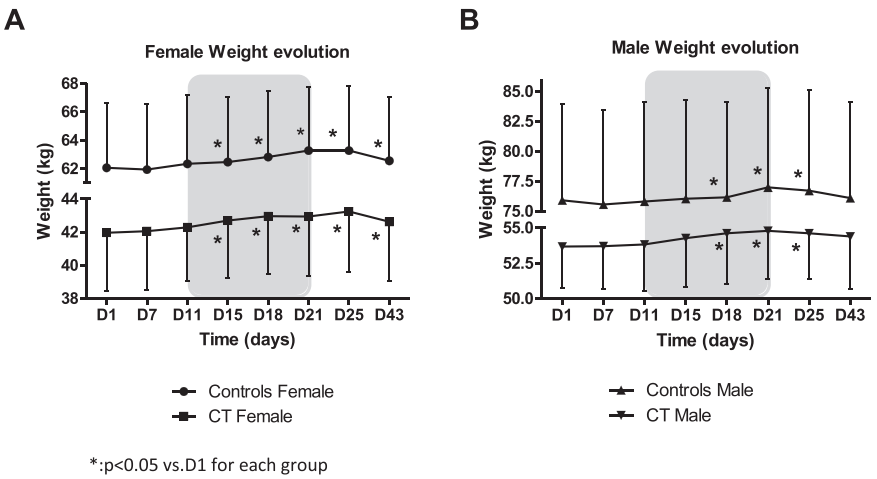

Figure 2 Weight changes in all groups throughout the study. Data are expressed as mean ± SD. *$P < 0.05$ vs. D1 in each group.

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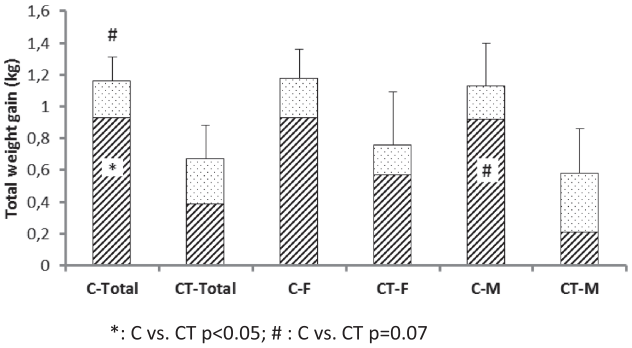

Figure 3 Total body weight, lean mass, and fat mass gain comparison. Data are presented as mean ± SD; hatched bars—lean mass; dotted bars—fat mass. Statistical analysis: *C vs. CT $P < 0.05$; #C vs. CT $P = 0.07$.

0.68 ± 0.21, $P = 0.07$) but not when considering each gender subgroup separately (*Figure* 3).

Body composition analyses revealed that LM significantly increased in controls in both genders but not in CTs. A trend to increase was noticed in CT women ($P = 0.07$). Overall calculated LM gain (LM_{V8}–LM_{V2}) was significantly higher in controls than in CT (0.93 ± 0.16 vs. 0.39 ± 0.19 kg, $P = 0.04$) and trend to be higher male controls (0.92 ± 0.26 vs 0.21 ± 0.26 kg, $P = 0.07$). FM increased significantly in both groups but no differences in calculated FM gain (FM_{V8}–FM_{V2}) were found between groups.

Total energy intake increased significantly during the overfeeding period in all study groups and study conditions. TEE remained stable in all groups in free-living conditions. Explored in the chamber stay, TEE significantly increased in controls only. As a result, energy gap was significantly increased by the overfeeding regimen in both CTs and controls (*Table* 2 and *Figure* 1).

In both study conditions, protein intake significantly increased in all groups, and the intake per bodyweight remained significantly higher in CTs compared to controls. Nitrogen losses were significantly increased in CTs subsequent to the intervention, yet the values remained lower to those of controls. The intervention affected strongly the nitrogen balance in controls, with significant positive changes. In these conditions the nitrogen balance became similar between CTs and controls (*Table* 3).

The overfeeding regimen shifted the preferential substrate oxidation at fasting state in CT women and in Controls by increasing carbohydrate oxidation rate (average of 1.35-fold increase) and decreasing fat oxidation (average of 0.84-fold decrease).

Fasting total and acylated ghrelin, PYY and GLP-1 levels were similar between CTs and controls before and after overfeeding period (*Table* 4 and *Figure* 4). The overfeeding intervention did not affect these hormonal parameters within each group. Test meal induced an acute postprandial fall of total and acylated ghrelin, similar in CTs and controls at baseline. The overfeeding intervention decreased significantly the mean total $ghrelin_{0\text{-}150min}$ in CTs and controls. Mean $PYY_{0\text{-}150min}$ and mean $GLP\text{-}1_{0\text{-}150min}$ were significantly elevated in CTs as compared with controls before and after overfeeding. Likewise, we observed that the intervention tended to induce an early secretory response of PYY to the test meal in CTs.

Urinary metabolomics identifies CT-specific metabolism (Figure 5)

Orthogonal partial least squares discriminant analysis models on total urine content normalization described metabolic differences between CT and controls at baseline. CT vs. controls differences in metabolome remained but were attenuated during the overfeeding period. Similar observations were achieved with data normalized to creatinine. Major/essential amino acids and central energy metabolism intermediates were present in higher concentrations in 24 h urine samples in CT compared with control subjects. The urinary metabolic phenotypic differences were not affected by the intervention.

Discussion

In the present study, we describe how the CT condition is characterized by a non-malnourished state of underweight phenotype in females[1,4,13] but also in male subjects. Indeed, both genders of CT exhibited no eating disorders traits, normal values of nutritional biomarkers (IGF-1, free T_3), and no excessive daily physical activity. Besides, both genders of CT displayed a lower percentage of body fat mass as compared with normal-weight controls, yet the values remained in the healthy range of body fat.[31] In line with our previous study,[13] CT's eating behaviour was associated in both genders to higher daily snacking episodes which accounted for non-negligible caloric contribution of snacking in TEI.

We report a paradoxical positive energy gap in CT male and female participants, confirming previous findings in women,[13] and discuss this phenotypic trait in relation to dietary, biochemical, and energy factors. No misreporting in food intake was found in our study, except for the common diet underreporting in normal-weight women in line with usual restrained eating scores as compared with a previous study.[2] The energy gap observed in CT individuals does not seem to be subject to a potential bias from our study design. This gap, shown in free-living conditions, was also confirmed in a controlled setting in CT women by using calorimetric chambers assessment. Taken all this, and in accordance with our usual energy equations, this paradoxical positive energy gap related to this particular CT phenotype might be necessary to prevent them from losing weight. Overfeeding ultimately led to weight gain in the CTs, although nominally

Table 4 Fasting and postprandial profiles of appetite-regulatory hormones at baseline and at post-overfeeding

Parameters		Baseline			Post-overfeeding				
		CTs	Controls	*P* value	CTs	Controls	*P* value	Time *P* value (CTs)	Time *P* value (controls)
Fasting total ghrelin (pg/mL)	Female	267.2 ± 116.7	237.4 ± 167.3	0.6406	274.5 ± 173.0	207.3 ± 116.9	0.2836	0.3882	0.4815
	Male	234.0 ± 160.6	182.0 ± 143.1	0.4449	196.5 ± 112.3	168.1 ± 124.2	0.5891	0.2051	0.4757
	Total	249.8 ± 138.9	212.2 ± 155.7	0.4093	235.5 ± 147.8	189.5 ± 119.0	0.2621	0.1218	0.3470
Fasting acylated ghrelin (pg/mL)	Female	90.7 ± 40.1	89.4 ± 57.2	0.9530	104.0 ± 62.9	93.1 ± 57.8	0.6687	0.9257	0.7225
	Male	88.8 ± 56.4	69.8 ± 60.3	0.4642	79.4 ± 59.3	62.3 ± 22.1	0.4024	0.3438	0.6721
	Total	89.7 ± 48.1	80.5 ± 58.1	0.5750	91.7 ± 60.9	79.1 ± 46.9	0.4470	0.4596	0.8818
Fasting PYY (pmol/mL)	Female	34.7 ± 22.5	33.2 ± 15.8	0.8527	47.3 ± 15.2	40.3 ± 20.8	0.3755	0.0840	0.2075
	Male	40.5 ± 19.7	29.6 ± 15.3	0.1777	38.0 ± 15.6	40.3 ± 11.9	0.7098	0.6298	0.0837
	Total	37.6 ± 20.9	31.6 ± 15.3	0.2808	42.6 ± 15.8	40.3 ± 17.0	0.6418	0.3294	0.0303
Fasting GLP-1 (pmol/mL)	Female	10.2 ± 4.4	10.1 ± 3.4	0.9266	12.1 ± 7.4	10.7 ± 4.3	0.5980	0.1063	0.5367
	Male	12.2 ± 5.9	8.4 ± 2.4	0.0686	10.9 ± 4.5	9.7 ± 2.9	0.4743	0.5276	0.0955
	Total	11.2 ± 5.2	9.3 ± 3.0	0.1394	11.5 ± 6.1	10.2 ± 3.7	0.4107	0.4411	0.1381
Test meal mean Total ghrelin (pg/L)	Female	162.1 ± 112.7	141.0 ± 110.8	0.2404	144.6 ± 110.8	121.2 ± 81.6	0.1309	0.0029	0.0472
	Male	133.1 ± 103.2	118.9 ± 103.8	0.4059	118.1 ± 82.5	105.4 ± 75.0	0.3355	0.0172	0.0691
	Total	147.4 ± 108.6	130.8 ± 107.9	0.1829	131.2 ± 98.1	114.0 ± 78.8	0.0934	0.0001	0.0075
Test meal mean acylated ghrelin (pmol/mL)	Female	56.1 ± 49.1	53.2 ± 40.9	0.6941	54.8 ± 45.6	54.3 ± 43.3	0.9412	0.2715	0.8227
	male	49.7 ± 41.8	42.2 ± 40.6	0.2614	43.3 ± 41.8	40.1 ± 23.9	0.5705	0.0333	0.6177
	Total	52.9 ± 44.8	48.2 ± 41.0	0.3442	49.1 ± 44.0	47.8 ± 36.3	0.7823	0.0256	0.7933
Test meal mean PYY (pmol/mL)	Female	52.9 ± 28.5	46.9 ± 19.9	0.1180	51.2 ± 15.8	46.6 ± 20.0	0.1104	0.2449	0.8640
	Male	53.9 ± 26.7	45.6 ± 17.9	0.0299	51.4 ± 24.1	46.6 ± 14.3	0.1481	0.3006	0.6405
	Total	53.4 ± 27.6	46.3 ± 19.0	0.0089	51.3 ± 20.2	46.6 ± 17.6	0.0307	0.8833	0.9982
Test meal mean GLP-1 (pmol/L)	Female	17.5 ± 9.0	16.6 ± 7.7	0.4686	17.3 ± 7.7	15.1 ± 7.5	0.0656	0.6731	0.0043
	Male	19.0 ± 8.1	13.6 ± 5.0	0.0001	17.9 ± 8.4	13.1 ± 4.7	<0.0001	0.0705	0.3426
	Total	18.3 ± 8.6	15.2 ± 6.7	0.0006	17.6 ± 8.0	14.1 ± 6.4	<0.0001	0.1322	0.0047

Data are expressed as mean ± SD.
CT, constitutional thinness; GLP-1, glucagon-like peptide-1; PYY, peptide tyrosine tyrosine.

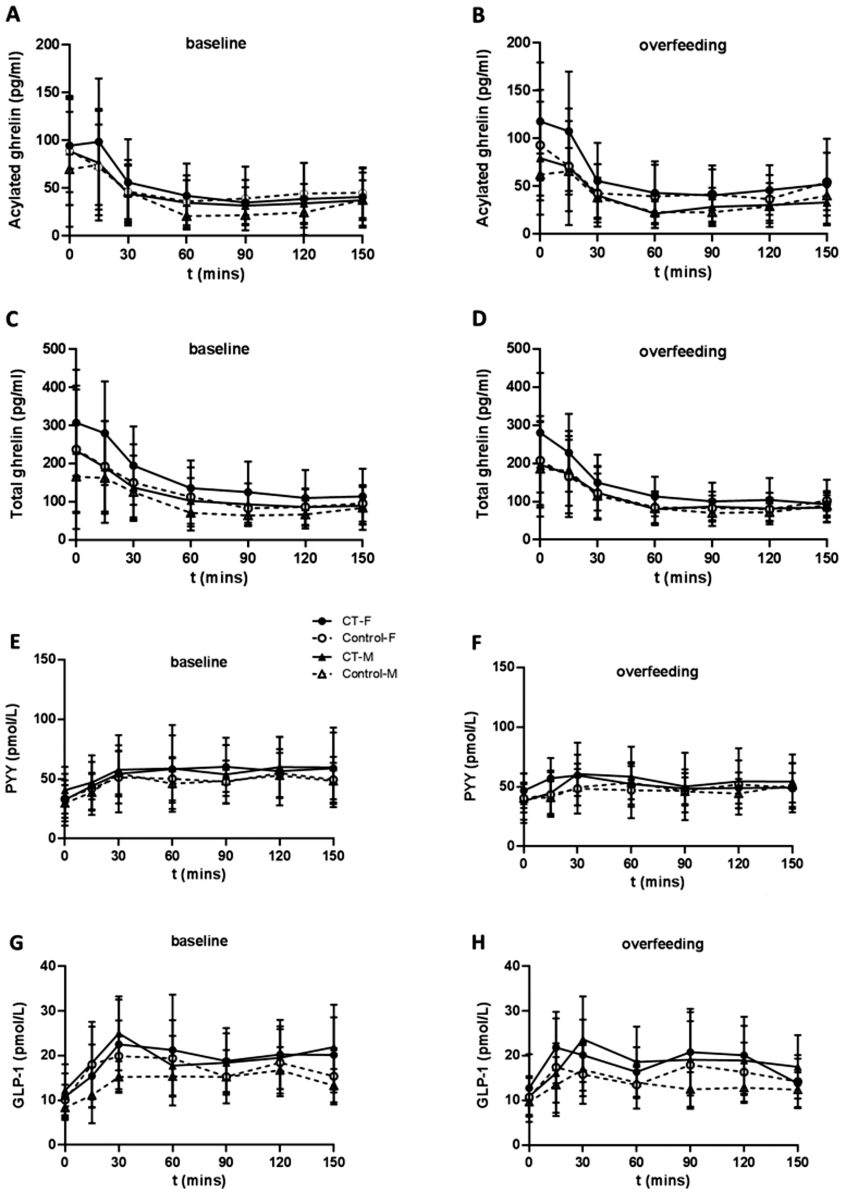

Figure 4 Post-test meal kinetic changes in appetite-regulatory hormones prior to overfeeding and at the end of the overfeeding in the constitutional thinness group (CT, dotted lines) and the control group (controls, plain lines). (A) Acylated ghrelin before and (B) after overfeeding; (C) Total ghrelin before and (D) after overfeeding; (E) PYY before and (F) after overfeeding; (G) GLP-1 before and (H) after overfeeding. Data are expressed as mean ± SD. Statistical analysis: $^{*}P < 0.05$ vs. T_0 min in each group. GLP-1, glucagon-like peptide 1; PYY, peptide tyrosine tyrosine.

lower than in the controls. This suggests that CTs' inability to gain weight might be overpassed at the cost of a particularly accentuated positive gap.

We further explored how this phenotype associates with distinct metabolic and energy states by considering the study of lipid, carbohydrate, and protein balance within basal and overfeeding conditions.[32]

Carbohydrate oxidation rate was higher in CT female subjects, which could be related to their higher food intake. The overfeeding intervention led to a decrease in fasting fat oxidation rate and increase in carbohydrate oxidation rate in all female subjects. This observation is in agreement with previous studies reporting a decrease in fat oxidation in obesity-prone subjects with overfeeding[33,34] and a shift to carbohydrate instead of lipid oxidation upon lipid overfeeding of healthy men.[34]

CTs manifested a positive nitrogen balance that was markedly higher than in controls. First, it is important to note that CTs still consume similar amounts of food as controls and in particular proteins even though the protein needs should to be lower given their lower lean body mass. The positive nitrogen balance was mainly due to a lower nitrogen excretion compared with controls, suggesting a saturated lean mass protein turn over. We indeed recently reported that skeletal

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A

Metabolites	^{1}H NMR chemical shift for variable	HMDB	Chisq2	p-value	Adjusted p-value
Alpha-Hydroxyisobutyric acid	1.36	HMDB0000729	7.37	0.00662	0.01924
Oxoglutaric acid	3.0	HMDB0000208	19.89	8.20E-06	0.0001804
3-Hydroxyisovaleric acid	1.27	HMDB0000754	7.47	0.00628	0.0186
Acetoacetic acid	2.30	HMDB0000060	16.3	5.40E-05	0.000693
L-Acetylcarnitine	2.14	HMDB0000201	11.9	0.00056	0.002782
L-Alanine	1.48	HMDB0000161	7.79	0.00525	0.01617
Allantoin	5.41	HMDB0000462	21.82	3.00E-06	9.24E-05
L-Arginine	1.78	HMDB0000517	7.18	0.00735	0.02058
Creatine	3.94	HMDB0000064	7.58	0.00589	0.01779
Dimethylamine	2.72	HMDB0000087	13.47	0.00024	0.001422
D-Glucose	5.25	HMDB0000122	16.46	5.00E-05	0.000693
L-Glutamic acid	2.37	HMDB0000148	23.31	1.40E-06	5.39E-05
L-Glutamine	2.45	HMDB0000641	16.38	5.20E-05	0.000693
Glycine	3.57	HMDB0000123	14.46	0.00014	0.001078
L-Isoleucine	0.95	HMDB0000172	14.85	0.00012	0.001054
L-Lactic acid	4.12	HMDB0000190	13.35	0.00026	0.001483
L-Leucine	0.96	HMDB0000687	17.62	2.70E-05	0.000462
Methylmalonic acid	1.26	HMDB0000202	10.98	0.00092	0.003978
myo-Inositol	3.52	HMDB0000211	26.66	2.40E-07	1.23E-05
N acetyl glycoprotein	2.07	NA	14.58	0.00013	0.001054
O acetyl glycoproteins	2.08	NA	8.01	0.00465	0.01492
Phosphocreatine	3.96	HMDB0001511	15.38	8.80E-05	0.0009625
Taurine	3.44	HMDB0000251	12.02	0.00053	0.002772
L-Threonine	4.24	HMDB0000167	29.73	5.00E-08	6.39E-06
L-Tryptophan	7.28	HMDB0000929	7	0.00813	0.02135
L-Tyrosine	7.20	HMDB0000158	6.13	0.01331	0.03254
L-Valine	1.00	HMDB0000883	13.46	0.00024	0.001422

B

Metabolite s	Fold of change between CT/control				
	Visit 1	Visit 3	Visit 6	Visit 7	Visit 8
2-alpha-hydroxyisobutyrate	1.05	1.06	1.01	1.06	1.09
2-oxoglutaric acid	1.10	1.14	1.13	1.16	1.16
3-hydroxyisovaleric acid	1.16	1.16	1.13	1.26	1.03
Acetoacetate	1.15	1.13	1.17	1.23	1.17
Acetyl-carnitine	1.15	1.17	1.26	1.24	1.05
Alanine	1.05	1.13	1.29	1.26	1.10
Allantoine	1.33	1.08	1.37	1.26	1.33
Arginine	1.14	1.12	1.16	1.12	1.14
Creatine	1.06	0.98	1.19	1.43	1.68
Dimethylamine	1.07	1.16	1.11	1.14	1.18
Glucose	1.24	1.27	1.26	1.31	1.14
Glutamate	1.08	1.17	1.16	1.22	1.13
Glutamine	1.10	1.13	1.20	1.21	1.10
Glycine	1.37	1.44	1.48	1.43	1.22
Isoleucine	1.11	1.15	1.10	1.13	1.20
Lactate	1.02	1.04	1.29	1.16	1.21
Leucine	1.13	1.21	1.11	1.18	1.18
Methylmalonate	1.10	1.19	1.13	1.20	1.14
Myo-inositol	1.23	1.20	1.21	1.20	1.13
NAC	1.18	1.10	1.16	1.15	1.06
OAC	1.17	1.13	1.19	1.13	1.06
Phospho creatine	1.05	1.15	1.11	1.24	1.12
Taurine	1.20	1.30	1.14	1.20	1.11
Threonine	1.22	1.20	1.13	1.16	1.19
Tryptophane	1.08	1.23	1.08	1.23	1.17
Tyrosine	0.93	1.28	1.13	1.23	1.24
Valine	1.17	1.15	1.14	1.16	1.12

C

	V2,V3 (baseline)	V6,V7,V8 (during overfeeding)
Unnormalized		
R^2X	0.16-0.17	0.14-0.18
R^2Y	0.61-0.66	0.53-0.67
Q^2Y	0.20-0.21	<0-0.11
Normalized to creatinine		
R^2X	0.37-0.54	0.48-0.52
R^2Y	0.46-0.52	0.39-0.44
Q^2Y	0.11-0.25	<0-0.13

Figure 5 Urinary metabolomics analyses: (A) urinary metabolite fold of change between CT and control subjects at the different visits; (B) results from analysis of variance on urine metabolite/creatinine ratios for a main effect of CT status; (C) parameters describing the orthogonal partial least squares discriminant analysis model, generated with one predictive and one orthogonal component, on total urine content. R2X is the explained variance in the urine metabolic profile, R2Y the explained group variance and Q2Y an indicator of mode robustness.

muscle energy metabolism was altered in CT women including a downregulation of cytoskeleton proteins and those involved in triglyceride storage or respiratory metabolism.[15] Oppositely to undernutrition or other catabolic situations associating a negative nitrogen balance,[35,36] a possible saturated protein turn over condition could lead in CTs to higher rates of amino acids flux. The findings generated through analysis of 24 h urine metabolomics provided evidence supporting this hypothesis. Indeed, we found higher urinary content in some essential amino acids including branched chain amino acids such as isoleucine, leucine, and valine but also threonine or tryptophan. Higher urinary levels of alanine and glutamine, both synthetized from branched chain amino acids in skeletal muscle and subsequently higher levels of arginine[37] suggest a global overload of protein turn over. Further physiological studies (especially with emphasis on total protein turn over) could provide deeper insights into the role of futile cycles in the CT phenotype. In particular, this metabolic trait seems to occur independently of overfeeding conditions and not to influence specific metabolic adaption to overfeeding. Hence, skeletal muscle inefficiency might play a role for some energy loss that could be part of gap explanations. Altogether, we assumed that this particular skeletal muscle phenotype could be a susceptive factor of CTs inability to gain lean mass contrary to controls after the overfeeding regimen. While this latter result should be interpreted with caution giving the small number of subjects some other data strengthen the concept of resistance to lean mass gain in CT. Indeed, CTs maintain a low lean mass in basal conditions, despite a high relative protein intake with more than 1.6 g/kg/day of protein intake, almost twice as the protein dietary intake recommendations in adults (0.8 g/kg/day).[38] This value is also higher than controls protein intake (1.2 g/kg/day) in our study, a value which fits the protein requirements in adults estimated by using an indicator amino acid oxidation technique.[39] CTs were still unable to increase their lean mass after overfeeding, whereas protein intake was raised to approximately 2.2 g/kg/day. These findings should be taken into account in clinical practice. While diagnosing sarcopenia in CT ageing patients or misdiagnosing CT as sarcopenia, a hypercaloric hyperprotein diet might be inefficient. We are currently evaluating whether an approach centred on physical activity could be useful to protect muscles in this particular population.

This high-protein consumption profile was associated with the postprandial anorectic tone found in CTs, in complete mirror image of obesity.[40,41] We confirmed, in both gender, a higher postprandial tone of PYY and GLP-1. We could

assume that this specific hormonal profile could be due to the high-protein consumption profile. This could be interesting to challenge CT patients by decreasing their protein intake in order to test if we could decrease PYY and GLP-1 postprandial rise. This hormonal and nutritional profile in CT could account for higher satiety feeling[42] and specific eating behaviour including smaller meals and frequent snacking[43] that should be interpreted as adaptive in this population.

Some limitations are raised by our study. Compliance to overfeeding protocol could be a major concern in this outpatient study. Currently, there is no reliable method to measure food intake in humans. Strict and frequent supervision by a dietician was therefore completed to ensure accurate food intake data and no over reporting was noted. It is very interesting to note that male participants appeared to be less prone than women to comply with the diet. Some indirect markers such as protein plasma level and nitrogen balance were used to check for accuracy of food intake reporting. Only participants who really complied with the overfeeding diet were included in statistical analyses using strict criteria. The subsequent small number of the participants may explain the lack of power in some results especially when analysis was performed for each gender.

To conclude, the blunted muscle energy mechanism, previously described in CTs in free-living state, is associated with basal saturated protein turn over suggested by the concordance of positive nitrogen balance and an increased urine excretion of essential amino acids. Most interestingly the fact that this saturation cannot be overpassed when enhancing this spontaneous high-protein intake supports the concept of the resistance to lean mass gain in this phenotype. Specific isotopic studies on amino acids turn over are needed to deeper explore this uncommon protein metabolism profile. CT patients profile should be taken into account all over their lives in order to avoid ineffective, aggressive therapies especially when clinical diagnosis of age related sarcopenia is evoked.

Author contributions

Bogdan Galusca, Jorg Hager, Nele Gheldof, Bruno Estour, and Natacha Germain carried out the coordination and design of the study; Yiin Ling, Bogdan Galusca, Jacques Epelbaum, Dominique Grouselle, Yves Boirie, Christophe Montaurier, Joyceline Cuenco, James S. Minnion, Thierry Thomas, Sylvie Mure, Simona Bartova, Sofia Moco, Francois-Pierre Martin, Nele Gheldof, and Natacha Germain performed the data collection; Yiin Ling, Bogdan Galusca, and Jerome Carayol performed the statistical analysis; Yiin Ling, Bogdan Galusca, Bruno Estour, Francois-Pierre Martin, Nele Gheldof, and Natacha Germain carried out the manuscript writing. All authors took part in the writing and final editing of the manuscript. All authors have been given a copy of the manuscript, all have approved the final version of the manuscript, and all are prepared to take public responsibility for the work and share responsibility and accountability for the results.

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Online supplementary material

Additional supporting information may be found online in the Supporting Information section at the end of the article.

Data S1 Supporting Information
Data S2 Supporting Information

Conflict of interest

François-Pierre Martin, Jérome Carayol, Simona Bartova, Sofia Moco, Jorg Hager, and Nele Gheldof are employees of Nestlé SA. Yiin Ling, Bogdan Galusca, Jacques Epelbaum, Dominique Grouselle, Yves Boirie, Christophe Montaurier, Joyceline Cuenco, James S. Minnion, Thierry Thomas, Sylvie Mure, Bruno Estour, and Natacha Germain declare that they have no conflict of interest.

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