Received: 11 November 2021 | Revised: 14 April 2022 | Accepted: 13 May 2022

DOI: 10.1002/prca.202100114

Proteomics
Clinical Applications

RESEARCH ARTICLE

Proteomics reveals unique plasma signatures in constitutional thinness

Ornella Cominetti[1] | Antonio Núñez Galindo[1] | John Corthésy[1] | Jérôme Carayol[2] | Natacha Germain[3] | Bogdan Galusca[3] | Bruno Estour[3] | Jörg Hager[2] | Nele Gheldof[2] | Loïc Dayon[1,4]

[1]Nestlé Institute of Food Safety & Analytical Sciences, Nestlé Research, Lausanne, Switzerland

[2]Nestlé Institute of Health Sciences, Nestlé Research, Lausanne, Switzerland

[3]Division of Endocrinology, Diabetes, Metabolism and Eating Disorders, CHU St-Etienne, France

[4]Institut des Sciences et Ingénierie Chimiques, École Polytechnique Fédérale de Lausanne (EPFL), Lausanne, Switzerland

Correspondence
Ornella Cominetti and Loïc Dayon. Proteomics, Nestlé Institute of Food Safety & Analytical Sciences, Nestlé Research, EPFL Innovation Park, Bâtiment H, 1015 Lausanne, Switzerland.
Emails: Ornella.Cominetti@rd.nestle.com and Loic.Dayon@rd.nestle.com

Present address
Nele Gheldof, VPA - AVP-R-Administration, EPFL, BI A2 483, Station 7, Lausanne 1015, Switzerland
Jérôme Carayol, Playtika Switzerland SA, Rue du Port-Franc 2A, Lausanne 1003, Switzerland

Abstract

Purpose: Studying the plasma proteome of control versus constitutionally thin (CT) individuals, exposed to overfeeding, may give insights into weight-gain management, providing relevant information to the clinical entity of weight-gain resistant CT, and discovering new markers for the condition.

Experimental Design: Untargeted protein relative quantification of 63 CT and normal-weight individuals was obtained in blood plasma at baseline, during and after an overfeeding challenge using mass spectrometry-based proteomics.

Results: The plasma proteome of CT subjects presented limited specificity with respect to controls at baseline. Yet, CT showed lower levels of inflammatory C-reactive protein and larger levels of protective insulin-like growth factor-binding protein 2. Differences were more marked during and after overfeeding. CT plasma proteome showed larger magnitude and significance in response, suggesting enhanced "resilience" and more rapid adaptation to changes. Four proteins behaved similarly between CT and controls, while five were regulated in opposite fashion. Ten proteins were differential during overfeeding in CT only (including increased fatty acid-binding protein and glyceraldehyde-3-phosphate dehydrogenase, and decreased apolipoprotein C-II and transferrin receptor protein 1).

Conclusions and Clinical Relevance: This first proteomic profiling of a CT cohort reveals different plasma proteomes between CT subjects and controls in a longitudinal clinical trial. Our molecular observations further support that the resistance to weight gain in CT subjects appears predominantly biological.

ClinicalTrials.gov Identifier: NCT02004821

Abbreviations: AGC, automatic gain control; BMI, body mass index; CRP, C-reactive protein; CT, constitutional thinness; EDTA, ethylenediaminetetraacetic acid; FA, formic acid; FC, fold change; HCD, higher-energy collisional dissociation; IAA, iodoacetamide; LACB, β-lactoglobulin; LC, liquid chromatography; MARS, multiple affinity removal system; MS, mass spectrometry; MS/MS, tandem mass spectrometry; RP, reversed-phase; SCX, strong cation-exchange; TCEP, tris(2-carboxyethyl) phosphine hydrochloride; TMT, tandem mass tag; V1, visit 1 (at baseline, before overnutrition); V6, visit 6 (during overnutrition); V9, visit 9 (11 days after the overfeeding intervention).

This is an open access article under the terms of the Creative Commons Attribution-NonCommercial-NoDerivs License, which permits use and distribution in any medium, provided the original work is properly cited, the use is non-commercial and no modifications or adaptations are made.
© 2022 The Authors. Proteomics – Clinical Applications published by Wiley-VCH GmbH.

KEYWORDS
constitutional thinness, isobaric tagging, mass spectrometry, overfeeding challenge, shotgun proteomics

1 | INTRODUCTION

Constitutional thinness (CT) is characterized by a stable natural state of low body weight with a differential diagnosis of anorexia nervosa [1, 2]. CT individuals (so called CTs in the following) do not have any eating or psychological disorder, food restriction, or hormonal signs of undernutrition, and females show normal physiological menses [3]. CTs present low average percentage of body fat, but not as low as anorexics; their leptin levels are low, but not excessively low [4]. Furthermore, their energy balance appears to be stable yet their profile of appetite regulating hormones resembles more that of normal-weight subjects than that of anorexic subjects [4, 5]. CT remains to date poorly studied and described. Its definition and diagnosis are yet to be well harmonized [3].

The CT phenotype is relevant as it should help understand the mechanisms to weight-gain resistance but could also be a key to help manage weight regain after weight loss. There are several known factors associated with weight regain after successful weight loss in obesity, from physiological (hormonal, appetite regulation, and energy expenditure-related, among others) to behavioral and psychological factors. Maintaining weight loss or weight gain is associated with compensatory changes in energy expenditure [6]. Alteration of circulating mediators of appetite is partly responsible for weight regain after diet-induced weight loss [7, 8]. From a psychosocial point of view, weight loss maintenance has been linked to comfort eating, reward eating, and stress. Such additional factors associated with weight regain or reduction are meal patterns, physical activity, and instrumentalization of eating [9].

Plasma proteome profiling refers to the semiquantitative determination of hundreds to thousands of proteins that circulate in the liquid portion of the blood. Blood is the main matrix used in the clinical laboratory and its biomarkers can support the diagnosis, prognosis, monitoring, and classification of subjects, for instance. Proteins constitute an important, if not the main, class of measured circulating biomarkers. Yet, their determination is today often discrete and sequential. The comprehensive nature of measurements offered by proteomic technologies may represent a paradigm shift that could pave the way to enhanced phenotyping in health and disease and translate into the clinics.

Among proteomic technologies, mass spectrometry (MS) is well established as a gold standard. MS-based profiling of human plasma has been deployed in the last decade to larger and larger clinical research studies [10–13] and has revealed valuable to identify candidate biomarkers and pinpoint biological and peripheral mechanisms, forecasting its potential for applicability and utility in clinical practices [14, 15].

In the present work, we aimed at investigating the specific plasma proteome of CT using state-of-the-art MS-based proteomics and revealing its molecular characteristics. Not only CT individuals were compared to normal-weight healthy subjects, but the study was carried out longitudinally to reveal fine differences between the phenotypes. An overfeeding challenge of 2 weeks was previously designed to confirm a positive energy balance in CT patients, as well as to attempt to find mechanistic explanations for weight resistance [16]. Such challenge may also be used to guide the development of a therapeutic approach to aid CT patients to gain weight but also to provide insights into the opposite situation of resistance to weight loss or weight regain in obese patients [16].

2 | EXPERIMENTAL SECTION

2.1 | Clinical study

The detailed clinical study design can be found in ref. [17] and was undertaken at the Centre Hospitalier Universitaire of Saint-Étienne (St-Etienne, France). The study was comprised of 32 CT subjects (15 females and 17 males; body mass index [BMI] $\leq$ 18.5 kg/m^2) and 31 normal-weight healthy controls (17 females and 14 males; BMI from 20 to 25 kg/m^2) matched for age (18–35 years) and physical activity levels.

Inclusion criteria common to both groups were: 18 and 35 years of age at the inclusion visit; normal blood sugar, liver function, lipid, and coagulation profiles; acceptance of a moderate weight gain of 2 kg. Inclusion criteria for CT subjects included: BMI less or equal to 17.5 (women) or 18.5 (men) and stable weight for at least 3 months. Inclusion criteria for control subjects included: BMI above or equal to 20 but not more than 25 (women and men), stable weight for at least 3 months and no previous family history of first or second-degree obesity [17].

Exclusion criteria for all individuals included: pregnancy in the case of women; women without contraception; vegetarian subjects or those with lactose intolerance; subjects with an eating disorder (classified using so-called DSM-IV); significant alcohol consumption equivalent to more than 10 glasses of wine per week; severe progressive disorder (e.g., diabetes); subjects who undertake intensive physical activity (more than three sessions of physical activity per week); significant tobacco consumption equivalent to more than 10 cigarettes per day; depression or psychiatric condition (treated with antidepressant or psychotropic medication); medical or surgical history considered by the investigators to be incompatible with the study (e.g., stomach or intestinal surgery); subjects receiving treatment

that may interfere with the parameters measured such as antihypertensives (blockers, centrally acting antihypertensives), antihyperlipidemic agents, or corticosteroids for more than 8 days; subjects having participated in a different clinical study within the previous 12 weeks [17].

Blood samples were obtained before (visit 1, at baseline, before overnutrition,V1), during (visit 6, during overnutrition, V6), and 11 days after the overfeeding intervention (visit 9, 11 days after the overfeeding intervention, V9). During the 14-consecutive days of the intervention, all subjects consumed each day a full bottle of Renutryl Booster (Nestlé Health Science, Vevey, Switzerland) composed of 72 g carbohydrates (48.5%), 30 g proteins (20%), and 21 g fat (31.5%), which provided an additional 600 kcal to their daily calorie intake. The study protocol was performed according to the Declaration of Helsinki, approved by the Ethics Committee France: ANSM (2013-A00590−45), and registered at clinical-trials.gov as NCT02004821.

Clinical Relevance

Plasma proteomic profiling using mass spectrometry has confirmed its potential for biomarker discovery and use in health and disease phenotyping. Longitudinal studies allow individuals to act as their own controls and are informative and very complementary to large sample-size case-control study designs.

Constitutional thinness (CT) is a condition in which subjects have a natural low body weight (body mass index ≤ 18 kg/m^2), without eating or psychological disorders, food restriction, or hormonal signs of undernutrition. CT is poorly understood and studied, and there is very little known about the specific proteomes of CT.

A better understanding of changes in the plasma proteome of people who are weight-gain resistant, during an overfeeding challenge could potentially provide new insights with the goal to improve weight management of obese or overweight but also healthy weight individuals.

This study is, to the best of our knowledge, the first one to investigate the plasma proteome of CT individuals and its response to an overfeeding challenge with respect to that of average weight individuals.

2.2 | Sample collection and randomization

Venous blood samples were collected in tubes containing aprotinin and ethylenediaminetetraacetic acid (EDTA). EDTA-plasma samples were aliquoted and randomized for proteomic analysis on four 96 well analytical plates. Parameters considered to block the randomization were collection visit, gender, age, and CT versus control status. Pools of plasma samples were also prepared. Two identical pool samples were included in each of the 6-plex isobaric tagging experiments (see below for details) and used as anchor samples between experiments to link and relatively quantify proteins in all plasma samples together. Pools of samples completed empty positions on the 96-well plates. The MS-based proteomic workflow has been fully described previously [18].

2.3 | Materials

Iodoacetamide (IAA), tris(2-carboxyethyl) phosphine hydrochloride (TCEP), triethylammonium hydrogen carbonate buffer 1 M pH 8.5, sodium dodecyl sulfate, and β-lactoglobulin (LACB) from bovine milk were purchased from Sigma (St. Louis, MO, USA). Formic acid (FA, 99%) and CH_3CN were from BDH (VWR International Ltd., Poole, UK). Hydroxylamine solution 50 wt% in H_2O (99.999%) was acquired from Aldrich (Milwaukee, WI, USA). H_2O (18.2 MΩ cm at 25°C) was obtained from a Milli-Q apparatus (Millipore, Billerica, MA, USA). Trifluoroacetic acid Uvasol was sourced from Merck Millipore (Billerica, MA, USA). The 6-plex isobaric tandem mass tags (TMTs) were purchased from Thermo Scientific (Rockford, IL, USA). Sequencing grade modified Lys-C/trypsin was procured from Promega (Madison, WI, USA). For immuno-affinity depletion of 14 abundant human proteins, multiple affinity removal system (MARS) columns, Buffer A, and Buffer B were obtained from Agilent Technologies (Wilmington, DE, USA). Oasis HLB cartridges (1cc, 30 mg) were acquired from Waters (Milford, MA, USA) and Strata-X 33u Polymeric reversed-phase (RP) and Strata-X-C 33u Polymeric strong cation-exchange (SCX) solid-phase extraction (SPE) cartridges (30 mg/1 mL) from Phenomenex (Torrance, CA, USA).

2.4 | Sample preparation

From 25 μL of each plasma sample (diluted in 75 μL of Buffer A containing 0.0134 mg/mL LACB and filtered with 0.22 μm filter plate from Millipore), 14 abundant plasma proteins were removed, following the manufacturer instructions, with MARS columns and high-performance liquid chromatography (LC) systems (Thermo Scientific, San Jose, CA, USA) equipped with an HTC-PAL (CTC Analytics, Zwingen, Switzerland) fraction collectors. After immuno-depletion, samples were snap-frozen and stored at −80°C. Buffer exchange was performed with RP cartridges mounted on a 96-hole holder and a vacuum manifold, as previously described [18]. Samples were subsequently evaporated with a vacuum centrifuge (Thermo Scientific) and stored at −80°C. Reduction with TCEP, alkylation with IAA, digestion with Lys-C/trypsin, 6-plex TMT labeling, sample pooling, and SPE purification (Oasis HLB and SCX) were performed on a 4-channels Microlab Star liquid handler (Hamilton, Bonaduz, Switzerland) according to a previously reported protocol [18]. The pooled 6-plex TMT-labeled samples were then evaporated to dryness before storage at −80°C.

2.5 | Reversed-phase liquid chromatography tandem mass spectrometry (RP-LC MS/MS)

The dried samples were dissolved in 500 μL H_2O/CH_3CN/FA 96.9/3/0.1 for RP-LC tandem MS (MS/MS). RP-LC MS/MS was performed in duplicate on two independent, but identical systems composed of an Orbitrap Fusion LumosTribrid mass spectrometer and an Ultimate 3000 RSLC nanosystem (Thermo Scientific). Proteolytic peptides (injection of 5 μL of sample) were trapped on an Acclaim PepMap 75 μm × 2 cm (C18, 3 μm, 100 Å) precolumn and separated on an Acclaim PepMap RSLC 75 μm × 50 cm (C18, 2 μm, 100 Å) column (Thermo Scientific) coupled to a stainless-steel nanobore emitter (40 mm, OD 1/32″) (Thermo Scientific). The column was heated to 50°C using a PRSO-V1 column oven (Sonation, Biberach, Germany). Peptide separation was performed with a gradient of mobile phase C (H_2O/CH_3CN/FA 97.9:2:0.1) and D (H_2O/CH_3CN/FA 19.92:80:0.08): from 6.3% to 11% D over 12 min, from 11% to 17.5% over 75 min, from 17.5% to 25.5% D over 42 min, and from 25.5% to 40% D over 28 min, with final elution (98% D) and equilibration (6.3% D) using a further 23 min. The flow rate was 220 nL/min with a total analysis time of 180 min. Data were acquired using a data-dependent method. A positive ion spray voltage of 1400 V and a transfer tube temperature of 275°C were set up. For MS survey scans in profile mode, the Orbitrap resolution was 120,000 at $m/z = 200$ (automatic gain control [AGC] target of 2×10^5) with an m/z scan range from 300 to 1500, RF lens set at 30%, and maximum injection time of 100 ms. For MS/MS with higher-energy collisional dissociation (HCD) at 35% of the normalized collision energy, AGC target was set to 1×10^5 (isolation width of 0.7 in the quadrupole), with a resolution of 30,000 at $m/z = 200$, first mass at $m/z = 100$, and a maximum injection time of 105 ms with Orbitrap acquiring in profile mode. A duty cycle time of 3 s (top speed mode) was chosen to maximize the number of precursor ions to be selected for HCD-based MS/MS. Ions were injected for all available parallelizable time. Dynamic exclusion was set for 60 s within a ±10 ppm window. A lock mass of $m/z = 445.1200$ was used.

2.6 | Protein quantitation and identification

The Thermo Scientific Proteome Discoverer software (version 1.4.0288) was used as data processing interface. Identification was performed against the human UniProtKB/Swiss-Prot database (October 26, 2015 release) including the LACB sequence (20,198 sequences in total). Mascot (version 2.4.2, Matrix Sciences, London, UK) was used as search engine. Variable amino acid modifications were oxidized methionine, deamidated asparagine/glutamine, and 6-plex TMT-labeled peptide amino terminus. 6-plex TMT-labeled lysine was set as fixed modification as well as carbamidomethylation of cysteine. Trypsin was selected as the proteolytic enzyme, with a maximum of two potential missed cleavages. Peptide and fragment ion tolerance were set to 10 ppm and 0.02 Da, respectively. All Mascot result files were loaded into Scaffold Q+ 4.7.2 (Proteome Software, Portland, OR, USA) to be further searched with X! Tandem (The GPM, thegpm.org; version CYCLONE (2010.12.01.1)). Both peptide and protein false discovery rates were fixed at 1%, with a two unique peptide criterion to report protein identification. Relative quantitative protein values were exported from Scaffold Q+ as Log_2 of the protein ratio fold changes (FCs) with respect to their measurements in the pool samples, that is, mean Log_2 values after isotopic purity correction but without normalization applied between samples and experiments. Calculation, statistics, and creation of figures were performed using the software environment R, versions 3.2.4 and 4.0.2 (http://www.r-project.org/) [19].

As a first technical validation of MS results, we compared the values of the insulin-like growth factor I (UniProtKB protein identification code *IGF1*, UniProtKB accession number P05019) measured using enzyme-linked immunosorbent assay (Figure A1). There, we see a moderate positive correlation between the MS and immunoassay measurements ($R = 0.62$, p-value = 4.9e-07).

The identified proteins were checked for confidence (i.e., number of peptides and protein sequence coverage and if present in both LC-MS/MS replicates or not) and against a reference list of canonical human plasma proteins [20]. From the total 999 proteins measured, a maximum of 5% of missing value per protein was accepted, giving a final data matrix with 417 proteins and with 99.67% of completeness. Then, 254 out of the total 77,145 values were imputed using single average. Additionally, we removed those proteins that did not appear in the reference plasma list at the time of the analysis and/or that were considered of lower confidence due to small number of identified peptides and limited protein sequence coverage in the current study. Additionally, depleted protein albumin (*ALBU*, P02768) was also filtered out.

After removal of extreme outliers and considering the subjects with missing visits, the total number of subjects with both data: for V1 and V6 there were 58 subjects; for V6 and V9 there were 59 subjects and for V1 and V9 there were 57 out of 66 subjects in total.

2.7 | Data analysis

We measured age and gender effect by fitting a linear model of the Log_2 values of protein ratio FCs against the confounding factors of age and gender to the data at each time point. Performing an analysis of variance with the car_2.0-26 R package to each model, we assessed the significance of the effect of these covariates after correcting the p-values using the Bonferroni method.

Proteins showing significant gender effect (Table A1) were stratified accordingly to assess the significance of the differences between CT individuals and controls for each protein using a Mann–Whitney U test. In the global model, we controlled both for gender and age to adjust for residual confounding, given the stringency in the criteria to select gender-affected proteins. In the gender-stratified models, we only controlled for age.

STRING (v11.0) [21] was used to identify known associations between proteins. Proteins were entered using their identifier and selecting "Homo sapiens" as organism.

TABLE 1 Summary statistics of individuals according to age, gender, BMI, weight, height, and groups (CTs versus controls)

	Controls		CT individuals	
	Females (17)	Males (14)	Females (15)	Males (17)
Age (years)	21.94 ± 2.79	23.43 ± 2.87	26.73 ± 5.02	23.00 ± 3.69
BMI (kg/m^2)	22.91 ± 1.07	22.84 ± 1.01	16.54 ± 0.70	17.29 ± 0.68
Weight (kg)	63.20 ± 4.79	74.52 ± 7.41	43.04 ± 4.35	54.01 ± 2.84
Height (cm)	166.05 ± 6.28	180.41 ± 6.83	161.14 ± 6.15	176.72 ± 4.26

BMI, body mass index; CT, constitutional thinness.

3 | RESULTS AND DISCUSSION

3.1 | Baseline cohort characterization and quantitative proteomic measurements: Plasma proteome profiles of CT subjects

Table 1 summarizes the distribution of participants per gender, age, BMI, weight, and height at baseline. Subjects were young, between 19 and 34 years of age, with BMI levels classifying them as normal weight for controls and underweight for CTs, without significant differences between age, weight, or height for each gender between CTs and controls.

Both female and male CTs had lower mean body weight, BMI, fat mass (absolute and relative values), lean mass (absolute value), and leptin levels than their control counterparts. Yet there was no difference in nutritional markers such as albumin, triglycerides, nonesterified fatty acids, glycerol, free triiodothyronine, and insulin-like growth factor I [17, 22]. All individuals were insulin-sensitive and showed normal glucose tolerance as previously reported [17, 22].

Weight for individuals at the different time points is shown in Figure A2. On average, by V9 only male controls did not lose the weight gained previously.

As known, gender can show a strong effect in plasma proteins. In this dataset, gender was strongly associated to the levels of several proteins, shown in Table A1. We consistently identified the following proteins to be differential between genders: sex hormone-binding globulin (*SHBG*, P04278), pregnancy zone protein (*PZP*, P20742), vitamin K-dependent protein S (*PROS1*, P07225), and neural cell adhesion molecule 1 (*NCAM1*, P13591). Complement factor D, also known as adipsin (*CFAD*, P00746), showed a strong gender difference at V6 only (Figure A3). No protein showed statistically significant effect for age.

When comparing controls against CTs at baseline (see Table A2 and Figures A4–A8), the proteins that showed a significantly higher level in controls were C-reactive protein (*CRP*, P02741) with a mean FC of 1.83, followed by coagulation factor XIII B chain (*F13B*, P05160; FC of 1.18) as well as angiogenin (*ANGI*, P03950) and complement factor I (*CFAI*, P05156) (both with FC of 1.11). Next, was complement factor H (*CFAH*, P08603; FC of 1.09). On the other hand, the proteins that were higher in CTs were insulin-like growth factor-binding protein 2 (*IBP2*, P18065; FC of 0.80) and 1 (*IBP1*, P08833; FC of 0.84), thyroxine-binding globulin (*THBG*, P05543; FC of 0.86), secreted phosphoprotein 24 (*SPP24*, Q13103; FC of 0.88), and carboxypeptidase N catalytic chain (*CBPN*, P15169; FC of 0.90). Very interestingly, the difference in levels of insulin-like growth factor-binding protein 2 and *CRP* was also consistent for all time points between CTs and controls, suggesting that these proteins may represent consistent markers of how these subjects differ under different conditions. The boxplots for these proteins can be found in Figure A4 where one can clearly see the difference between groups at all time points. In Figure A8, we show the boxplots for those proteins able to significantly separate CTs from controls only at baseline, namely complement factor I, carboxypeptidase N catalytic chain, thyroxine-binding globulin, angiogenin, and secreted phosphoprotein 24. In Figure A9, we show the boxplots of proteins differential between subjects only at V6 and V9, namely protein S100-A9 (*S10A9*, P06702), protein S100-A8 (*S10A8*, P05109), and dopamine beta-hydroxylase (*DOPO*, P09172).

Insulin-like growth factor-binding protein 2 is encoded by the leptin-regulated *IGFBP2* gene. Plasma levels of insulin-like growth factor-binding protein 2 are known to correlate with insulin sensitivity; a strong inverse association with risk of type 2 diabetes mellitus has been shown [23, 24]. Additionally, insulin-like growth factor-binding protein 2 concentrations are inversely correlated with BMI and lower adiposity [23, 25, 26], which is also supported by our current data. Higher levels of insulin-like growth factor-binding protein 2 systematically observed in the blood plasma of CTs may therefore be a good biomarker of their phenotype. This may warrant further research related to its role in weight control. *CRP* is a nonspecific marker of inflammation and as such high circulating levels are observed in obese subjects [27]. The lower CRP plasma levels found in CTs would indicate an overall lower level of systemic inflammation in these subjects. Consistently, transcriptomic analysis previously performed in adipose tissue of these subjects also showed interleukin-8 (IL-8) signaling pathway to be reduced in CTs, IL-8 being a key proinflammatory mediator [17].

Among the proteins only differential at baseline between CTs and controls, angiogenin and complement factor I were higher in controls. Serum angiogenin was previously found significantly elevated in the serum of overweight and obese subjects and correlated with BMI [28], as was plasma complement factor I in one of our recent studies [13]. Carboxypeptidase N catalytic chain, thyroxine-binding globulin, and secreted phosphoprotein 24 had increased plasma levels in CTs at baseline. Carboxypeptidase N catalytic chain protects the body from potent vasoactive and inflammatory peptides released into the circulation. In a recent study, its *CPN1* gene presented the strongest transprotein quantitative trait locus single nucleotide polymorphism

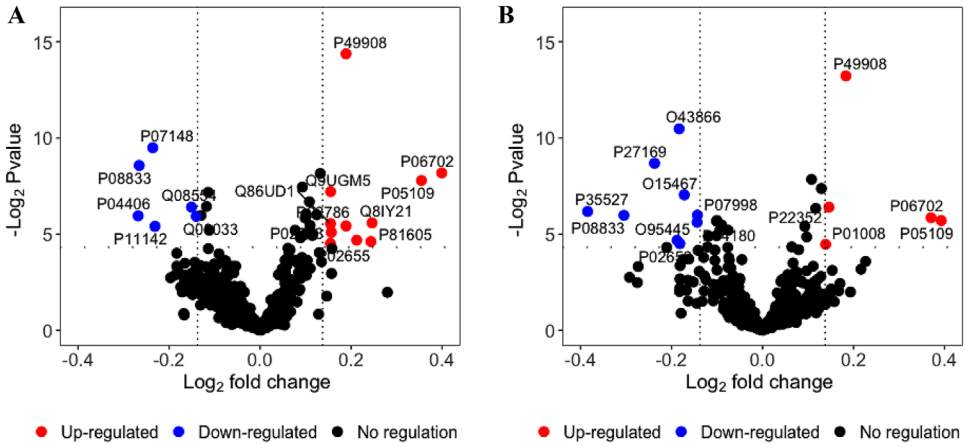

FIGURE 1 Volcano plot of plasma protein level changes between V6 and V1 in (A) CT subjects and (B) controls. Thresholds: *p*-value cut-off was fixed at 0.05, and FC cut-offs correspond to 1/1.1 and 1.1 (horizontal and vertical dashed lines, respectively). CT, constitutional thinness; FC, fold change; V1, visit 1 (at baseline, before overnutrition); V6, visit 6 (during overnutrition)

for calpastatin (*ICAL*, P20810) [29] an endogenous calpain inhibitor, involved in inflammatory processes, that is dysregulated in obesity [30]. Thyroxine-binding globulin is encoded by the *SERPINA7* gene and binds thyroid hormones in the circulation, the low level of which can lead to slower metabolism and weigh gain [31].

3.2 | Specific effects of an overfeeding challenge on the plasma proteome of CT subjects

In Figure 1, proteins are represented by their significance versus their FC (in logarithmic scale) in CTs and controls between the time points V6 and V1 (i.e., $\text{Log}_2(\text{Prot}^i_{V6}) - \text{Log}_2(\text{Prot}^i_{V1})$, where Prot^i_{vj} represents the average measurement of the *i*th protein at time point *j*), that corresponds roughly to the halfway point of the overfeeding challenge and the baseline, respectively. Proteins in red show significantly increased levels (*p*-value below 0.05) with an FC superior to 1.1 (10% – FC threshold), whereas proteins depicted in blue show significantly decreased levels with an FC below 1/1.1. These FC thresholds help identify proteins that have a higher potential as biomarkers, given that their differential measurement is relatively high when compared to the rest of proteins. In Figure A10, we summarized the significant *p*-values for both volcano plots into a single plot. There, one can directly compare the magnitude and number of proteins regulated. In our study, CTs showed a slightly larger number of proteins being regulated and with a much higher significance. The same was true, and even more markedly between V9 and V1, as seen in Figure A11.

Common upregulated proteins between V6 and V1 as a response to the overfeeding challenge for both CTs and controls were selenoprotein P (SEPP1, P49908), protein S100-A9, and protein S100-A8. There is conflicting evidence for relationship between selenoprotein P and glucose metabolism, but there is a clear association with thyroid function and selenoprotein P [32]. Glucose and insulin can regulate *SELENOP* gene expression in the liver and pancreas, and vice versa. S100-A8 and S100-A9 proteins make up the heterodimer calprotectin, which we found previously to be, at the opposite, downregulated during a weight loss and maintenance study in overweight and obese individuals [33]. Calprotectin is often found elevated in inflammatory disorders and low-grade inflammation conditions, including obesity, and as such is considered a marker for obesity in both males and females, in adults and children [34]. Detecting an increase of calprotectin levels following 2-week overfeeding in both CTs and controls appears therefore to be relevant to a certain extent. It shows an inflammatory response to the nutritional challenge, which is low-grade but not chronic (hence the absence of a strong *CRP* response) and may correspond to an inflammation in the gastrointestinal tract. Specific inflammatory response (systemic chronic low-grade in particular) has been previously shown to be induced by high-fat diets [35, 36].

The only common downregulated protein between V6 and V1 for both CTs and controls was insulin-like growth factor-binding protein 1. Insulin-like growth factor-binding proteins are known to be linked with overfeeding [37], obesity, and diabetes [23]. Bioavailable protein insulin-like growth factor I was inversely related to weight gain in a previous study [38], and its relation to insulin-like growth factor-binding protein 1 supports our findings (see Figure A1); yet protein insulin-like growth factor I response to overfeeding was not identified in our proteomic study.

Uniquely downregulated plasma proteins for CTs were liver fatty acid-binding protein (*FABPL*, P07148), heat shock cognate 71 kDa protein (*HSP7C*, P11142), glyceraldehyde-3-phosphate dehydrogenase (*G3P*, P04406), desmocollin-1 (*DSC1*, Q08554), and inter-alpha-trypsin inhibitor heavy chain H3 (*ITIH3*, Q06033). Uniquely upregulated plasma proteins for CTs were apolipoprotein C-II (*APOC2*, P02655), dermcidin (*DCD*, P81605), fetuin-B (*FETUB*, Q9UGM5), transferrin receptor protein 1 (*TFR1*, P02786), and out at first protein homolog (*OAF*, Q86UD1). Several of these proteins have been previously put in relation with obesity, weight gain and/or loss, and metabolic changes and alterations [33, 39–43]. In the transcriptomic analysis of adipose tissue of these subjects, oxidative stress factors (such

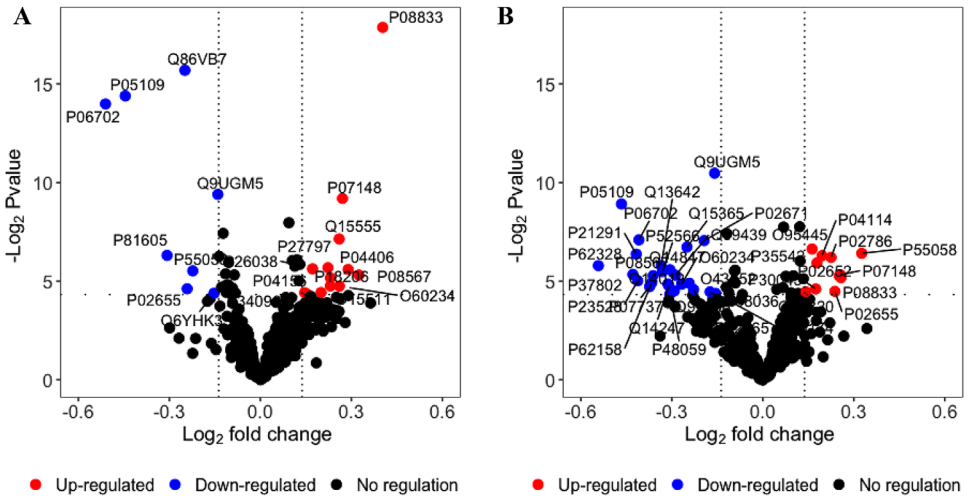

FIGURE 2 Volcano plot of plasma protein level changes between V9 and V6 in (A) CT subjects and (B) controls. Thresholds: *p*-value cut-off was fixed at 0.05, and FC cut-offs correspond to 1/1.1 and 1.1 (horizontal and vertical dashed lines, respectively). CT, constitutional thinness; FC, fold change; V9, visit 9 (11 days after the overfeeding intervention); V6, visit 6 (during overnutrition)

as *HSPB7* gene) were also found to be downregulated in CTs [17]. Their specific regulation and directionality of change in CTs during an overfeeding challenge may suggest more research is needed. *FABP1* and *ITIH3* genes have evidence of coexpression in humans (STRING v11.0) [21].

3.3 | Specific plasma protein changes during recovery after an overfeeding challenge in CT subjects

In Figure 2A, we observed that during the recovery phase, protein levels in CTs showed more significant and larger magnitude of changes than for controls. Specifically, the more significant and larger difference in protein level for CTs was found for insulin-like growth factor-binding protein 1, with a *p*-value of 4.17e-06 (equivalent to a $-\text{Log}_2$(*p*-value) of 17.87) and FC of 1.32. For controls, the strongest difference between V6 and V9 (among the proteins that passed the significance threshold) was for phospholipid transfer protein (*PLTP*, P55058) with an FC of 1.26 but not a strong significance (*p*-value = 0.012). The protein that showed the largest significance for the controls was fetuin-B, *p*-value = 7.05e-04, but its decreased FC was only 0.90.

A very interesting finding in this analysis was the appearance of phospholipid transfer protein, which was upregulated for controls between V6 and V9, but downregulated for CTs. The same opposite direction of change was true for apolipoprotein C-II. On the contrary, pleckstrin (*PLEK*, P08567) and glia maturation factor gamma (*GMFG*, O60234) were downregulated for controls between V6 and V9 but upregulated for CTs. Importantly, this was the first time we observed opposite protein level changes between CTs and controls and this appeared during the washout or recovery period between V6 and V9. Additionally, growth factor receptor-bound protein 2 (GRB2, P62993) increased for CTs (Table 2) but decreased during the recovery for controls (*p*-value = 0.0595) but did not pass the significance threshold. These proteins could shed light in the differences on how the CT subjects recover differently from an overfeeding challenge.

Common upregulated proteins between V6 and V9 for both CTs and controls were insulin-like growth factor-binding protein 1 (as it was commonly downregulated during the overfeeding phase) and liver fatty acid-binding protein. Common downregulated proteins between V6 and V9 for both CTs and controls were fetuin-B, protein S100-A9, and protein S100-A8. This may indicate that the decrease of the calprotectin complex mirrored the reduced inflammation during the washout period. Given that CRP did not show again marked changes, we may hypothesize that the individuals did not reach a low-grade systemic inflammation status, but that calprotectin as an early marker of onset of inflammatory reactions, was already giving indication of an altered inflammatory state in these subjects, which was temporary and reverted during the recovery phase.

Uniquely downregulated proteins between V6 and V9 for CTs were scavenger receptor cysteine-rich type 1 protein M130 (*C163A*, Q86VB7), dermcidin, and CD109 antigen (*CD109*, Q6YHK3). Decreased CD109 antigen was recently proposed as a novel putative marker of weight-loss and maintenance [33]. The decrease of CD109 antigen during the washout period in CT subjects may confirm this very first observation and warrant further investigations.

Uniquely upregulated proteins between V6 and V9 for CTs were microtubule-associated protein RP/EB family member 2 (*MARE2*, Q15555), glyceraldehyde-3-phosphate dehydrogenase (*GAPDH*, P04406), calreticulin (*CALR*, P27797), moesin (*MOES*, P26038), vinculin (*VINC*, P18206), actin-related protein 2/3 complex subunit 5 (*ARPC5*, O15511), ribonuclease 4 (*RNAS4*, P34096), and major prion protein (*PRIO*, P04156).

It is noticeable the larger amount of significantly downregulated proteins for controls versus CTs between V6 and V9 (25 versus nine

TABLE 2 Summary of protein differential abundances with directionality during the overfeeding challenge and recovery for CT individuals

UniprotKB accession	Protein name	Overfeeding (V6/V1) Level of change (Log_2 of FC)	Recovery (V9/V6) Level of change (Log_2 of FC)
P06702	Protein S100-A9	↑0.40	↓−0.51
P05109	Protein S100-A8	↑0.35	↓−0.45
P81605	Dermcidin	↑0.24	↓−0.31
Q86VB7	Scavenger receptor cysteine-rich type 1 protein M130	–	↓−0.25
P02655	Apolipoprotein C-II	↑0.21	↓−0.24
P55058	Phospholipid transfer protein	–	↓−0.22
P49908	Selenoprotein P	↑0.19	↓−0.12
P02786	Transferrin receptor protein 1	↑0.19	–
Q9UGM5	Fetuin-B	↑0.16	↓−0.14
Q6YHK3	CD109 antigen	–	↓−0.15
Q86UD1	Out at first protein homolog	↑0.15	–
P08833	Insulin-like growth factor-binding protein 1	↓−0.27	↑0.40
P08567	Pleckstrin	–	↑0.32
P04406	Glyceraldehyde-3-phosphate dehydrogenase	↓−0.27	↑0.29
P07148	Liver fatty acid-binding protein	↓−0.24	↑0.27
O60234	Glia maturation factor gamma	–	↑0.26
Q15555	Microtubule-associated protein RP/EB family member 2	–	↑0.26
P11142	Heat shock cognate 71 kDa protein	↓−0.23	–
O15511	Actin-related protein 2/3 complex subunit 5	–	↑0.23
P18206	Vinculin	–	↑0.23
P27797	Calreticulin	–	↑0.22
P62993	Growth factor receptor-bound protein 2	–	↑0.20
P26038	Moesin	–	↑0.17
Q08554	Desmocollin-1	↓−0.15	–
P04156	Major prion protein	–	↑0.15
P34096	Ribonuclease 4	–	↑0.15
Q06033	Inter-alpha-trypsin inhibitor heavy chain H3	↓−0.14	↑0.12

CT, constitutional thinness; FC, fold change; V1, visit 1 (at baseline, before overnutrition); V6, visit 6 (during overnutrition); V9, visit 9 (11 days after the overfeeding intervention).

proteins, respectively, represented as blue dots in Figure 2 for V9/V6, while for the overfeeding challenge, downregulated proteins were nine versus six, represented as blue dots in Figure 1 for V6/V1), suggesting differences in their metabolic flexibility was particularly manifest during the washout period. Such large number of proteins was not observed as significantly downregulated proteins when comparing V9/V1 (see Figures A12 and A13), or significant upregulated proteins when comparing V6/V1 neither for CTs or controls. This could occur if proteins presented a small increase during the first phase of the challenge and then a larger decrease during the recovery phase (Table A3), making the difference between V6 and V9 the most pronounced (hence the large number of downregulated proteins in the corresponding volcano plot in Figure 2B); yet differences between the protein levels at V9 and V1 were not large enough to appear as significant. This may suggest that control individuals had a more complex response than CTs in their metabolic adjustment, losing the gained weight. As a conclusion, weight loss (and not weight gain) may have a more pronounced impact in plasma protein levels within this challenge.

Among the four proteins whose intensities decreased during the challenge and then increased during recovery in CTs (Table 2), there is evidence for three of them having clear functional links (STRING v11.0) [21]: *FABP1* and *IGFBP1 genes* are coexpressed in humans. Likewise, *FABP1* and *ITIH3* also show evidence for coexpression in humans. The strongest coexpressions for these pairs in humans are *ITIH3* and *IGFBP1*. Apart from the components of calprotectin, no strong functional links were found for the seven proteins that showed the opposite trajectory (i.e., increasing during the challenge and decreasing during the recovery in CTs).

Altogether, we observed a reversibility in the proteome of the CT subjects, since most of the effects seen during the overfeeding phase

were reversed after the recovery phase, during the time frame studied. Potentially, after giving additional weeks to recover, additional proteins would have also reverted to their original levels (i.e., before the overfeeding challenge). In fact, seven out of the nine upregulated proteins in the overfeeding challenge were then downregulated during the recovery phase (Table 2). And out of the six downregulated proteins during the overfeeding phase, four were upregulated during the recovery phase (Table 2). Very interestingly and in line with our previous observations, for controls, this reversibility was slightly less marked. Three out of the five upregulated proteins during the overfeeding challenge were then downregulated during the recovery phase. And, out of the nine downregulated proteins during overfeeding, four were then upregulated during the recovery phase.

4 | CONCLUDING REMARKS

Overall, this study demonstrated for the first time the specificity of the plasma proteome of CT subjects with respect to that of normal-weight healthy controls, as well as the identification of two potential phenotypic biomarkers, namely CRP and insulin-like growth factor-binding protein 2. The extent of this proteome specificity was exacerbated by an overfeeding challenge and, markedly, during the following washout/recovery phase. Our observations further highlighted the enhanced flexibility of CTs to overnutrition. CTs showed a greater resistance to change, or "resilience" to change as observed in their plasma proteome, when compared to controls subjected to the same overfeeding intervention. The present findings also suggested there may exist different biological mechanisms, such as metabolic and inflammatory processes, between CTs and controls that transduce in distinctive plasma proteome landscapes.

Additional molecular mechanisms underlying the CT phenotype of the subjects described in the current work have been identified previously. Some mechanisms that could explain the weight resistance in CT are an overreaction of energy expenditure while overfeeding, which has been supported by changes in gut hormone profiles in CTs [44]. Additionally, and as observed before, CT presents a distinct skeletal muscle phenotype in which energy storage defects were present in muscle biopsies which may contribute in part to body-weight gain resistance. However, the reasons behind protein storage and turnover are not well understood in CT [22, 45]. A behavioral component which could explain the resistance to weight gain in CT could be explained by noncompliance in the overfeeding protocols, however, the difference in lipid plasma profiles of CT and controls, as well as metabolic trajectory changes in both groups suggest that CTs complied with the study protocol [44].

Adipose tissues have been characterized and it was found that despite the fat cells of CT subjects are smaller than in healthy individuals, they contain more mitochondria and present higher mitochondrial function. Distinct adipose transcriptomic profiles revealed altered lipid metabolism in CT, resulting in an increase of energy expenditure [17]. Taken together, these observations may help refine weight management strategies.

ACKNOWLEDGMENTS

We would like to thank Polina Mironova and Ondine Walter for their valued support with data management and biobanking and Martin Kussmann for his continuous support. We thank Irina Irincheeva for her work and support with sample randomization and experimental plate design. This research was funded by the Société des Produits Nestlé S.A.

CONFLICT OF INTEREST

Ornella Cominetti, Antonio Núñez Galindo, John Corthésy, Jérôme Carayol, Jörg Hager, Nele Gheldof, and Loïc Dayon are or were employed by the Société des Produits Nestlé S.A.

DATA AVAILABILITY STATEMENT

Data available on request due to privacy/ethical restrictions.

ORCID

Ornella Cominetti https://orcid.org/0000-0001-8564-1963
Antonio Núñez Galindo https://orcid.org/0000-0003-2619-4475
Jérôme Carayol https://orcid.org/0000-0002-0049-0244
Bogdan Galusca https://orcid.org/0000-0002-0154-6924
Nele Gheldof https://orcid.org/0000-0001-8922-7372
Loïc Dayon https://orcid.org/0000-0002-8499-270X

REFERENCES

1. Apfelbaum, M., & Sachet, P. (1982). Constitutional thinness. *La Revue Du Praticien*, *32*(3), 245–247.
2. Mitchell, J. E., Cook-Myers, T., & Wonderlich, S. A. (2005). Diagnostic criteria for anorexia nervosa: Looking ahead to DSM-V. *International Journal of Eating Disorders*, *37*, Suppl, S95–S97.
3. Bailly, M., Germain, N., Galusca, B., Courteix, D., Thivel, D., & Verney, J. (2020). Definition and diagnosis of constitutional thinness: A systematic review. *British Journal of Nutrition*, *124*(6), 531–547. https://doi.org/10.1017/S0007114520001440
4. Bossu, C., Galusca, B., Normand, S., Germain, N., Collet, P., Frere, D., Lang, F., Laville, M., & Estour, B. (2007). Energy expenditure adjusted for body composition differentiates constitutional thinness from both normal subjects and anorexia nervosa. *American Journal of Physiology.Endocrinology and Metabolism*, *292*(1), E132–E137. https://doi.org/10.1152/ajpendo.00241.2006
5. Germain, N., Galusca, B., Le Roux, C. W., Bossu, C., Ghatei, M. A., Lang, F., Bloom, S. R., & Estour, B. (2007). Constitutional thinness and lean anorexia nervosa display opposite concentrations of peptide YY, glucagon-like peptide 1, ghrelin, and leptin. *American Journal of Clinical Nutrition*, *85*(4), 967–971. https://doi.org/10.1093/ajcn/85.4.967
6. Lundgren, J. R., Janus, C., Jensen, S. B. K., Juhl, C. R., Olsen, L. M., Christensen, R. M., Svane, M. S., Bandholm, T., Bojsen-Møller, K. N., Blond, M. B., Jensen, J. - E. B., Stallknecht, B. M., Holst, J. J., Madsbad, S., & Torekov, S. S. (2021). Healthy weight loss maintenance with exercise, liraglutide, or both combined. *New England Journal of Medicine*, *384*(18), 1719–1730. https://doi.org/10.1056/NEJMoa2028198
7. Iepsen, E. W., Lundgren, J., Dirksen, C., Jensen, J.-E., Pedersen, O., Hansen, T., Madsbad, S., Holst, J. J., & Torekov, S. S. (2015). Treatment with a GLP-1 receptor agonist diminishes the decrease in free plasma leptin during maintenance of weight loss. *International Journal of Obesity*, *39*(5), 834–841. https://doi.org/10.1038/ijo.2014.177
8. Sumithran, P., Prendergast, L. A., Delbridge, E., Purcell, K., Shulkes, A., Kriketos, A., & Proietto, J. (2011). Long-term persistence of hormonal adaptations to weight loss. *New England Journal of Medicine*, *365*(17), 1597–1604. https://doi.org/10.1056/NEJMoa1105816

9. Christensen, B. J., Iepsen, E. W., Lundgren, J., Holm, L., Madsbad, S., Holst, J. J., & Torekov, S. S. (2017). Instrumentalization of eating improves weight loss maintenance in obesity. *Obesity Facts*, *10*(6), 633–647. https://doi.org/10.1159/000481138
10. Cominetti, O., Núñez Galindo, A., Corthésy, J., Oller Moreno, S., Irincheeva, I., Valsesia, A., Astrup, A., Saris, W. H. M., Hager, J., Kussmann, M., & Dayon, L. (2016). Proteomic biomarker discovery in 1000 human plasma samples with mass spectrometry. *Journal of Proteome Research*, *15*(2), 389–399. https://doi.org/10.1021/acs.jproteome.5b00901
11. Geyer, P. E., Kulak, N. A., Pichler, G., Holdt, L. M., Teupser, D., & Mann, M. (2016). Plasma proteome profiling to assess human health and disease. *Cell Systems*, *2*(3), 185–195.
12. Bruderer, R., Muntel, J., Müller, S., Bernhardt, O. M., Gandhi, T., Cominetti, O., Macron, C., Carayol, J., Rinner, O., Astrup, A., Saris, W. H. M., Hager, J., Valsesia, A., Dayon, L., & Reiter, L. (2019). Analysis of 1508 plasma samples by capillary-flow data-independent acquisition profiles proteomics of weight loss and maintenance. *Molecular & Cellular Proteomics*, *18*, 1242–1254. https://doi.org/10.1074/mcp.RA118.001288
13. Cominetti, O., Núñez Galindo, A., Corthésy, J., Valsesia, A., Irincheeva, I., Kussmann, M., Saris, W. H. M., Astrup, A., Mcpherson, R., Harper, M. E., Dent, R., Hager, J., & Dayon, L. (2018). Obesity shows preserved plasma proteome in large independent clinical cohorts. *Scientific Reports*, *8*, 16981. https://doi.org/10.1038/s41598-018-35321-7
14. Geyer, P. E., Holdt, L. M., Teupser, D., & Mann, M. (2017). Revisiting biomarker discovery by plasma proteomics. *Molecular Systems Biology*, *13*, 942. https://doi.org/10.15252/msb.20156297
15. Ignjatovic, V., Geyer, P. E., Palaniappan, K. K., Chaaban, J. E., Omenn, G. S., Baker, M. S., Deutsch, E. W., & Schwenk, J. M. (2019). Mass spectrometry-based plasma proteomics: Considerations from sample collection to achieving translational data. *Journal of Proteome Research*, *18*(12), 4085–4097. https://doi.org/10.1021/acs.jproteome.9b00503
16. Ling, Y., Galusca, B., Hager, J., Feasson, L., Valsesia, A., Epelbaum, J., Alexandre, V., Wynn, E., Dinet, C., Palaghiu, R., Peoc'h, M., Boirie, Y., Montaurier, C., Estour, B., & Germain, N. (2016). Rational and design of an overfeeding protocol in constitutional thinness: Understanding the physiology, metabolism and genetic background of resistance to weight gain. *Annales d'Endocrinologie*, *77*(5), 563–569. https://doi.org/10.1016/j.ando.2016.06.001
17. Ling, Y., Carayol, J., Galusca, B., Canto, C., Montaurier, C., Matone, A., Vassallo, I., Minehira, K., Alexandre, V., Cominetti, O., Núñez Galindo, A., Corthésy, J., Dayon, L., Charpagne, A., Métairon, S., Raymond, F., Descombes, P., Casteillo, F., Peo''h, M., … Gheldof, N. (2019). Persistent low body weight in humans is associated with higher mitochondrial activity in white adipose tissue. *American Journal of Clinical Nutrition*, *110*(3), 605–616. https://doi.org/10.1093/ajcn/nqz144
18. Dayon, L., Núñez Galindo, A., Corthésy, J., Cominetti, O., & Kussmann, M. (2014). Comprehensive and scalable highly automated MS-based proteomic workflow for clinical biomarker discovery in human plasma. *Journal of Proteome Research*, *13*, 3837–3845. https://doi.org/10.1021/pr500635f
19. R Core Team. (2016). *R: A language and environment for statistical computing*. R Foundation for Statistical Computing, Vienna, Austria.
20. Farrah, T., Deutsch, E. W., Omenn, G. S., Campbell, D. S., Sun, Z., Bletz, J. A., Mallick, P., Katz, J. E., Malmström, J., Ossola, R., Watts, J. D., Lin, B., Zhang, H., Moritz, R. L., & Aebersold, R. (2011). A high-confidence human plasma proteome reference set with estimated concentrations in peptide atlas, *Molecular & Cellular Proteomics*, *10*(9), M110.006353.
21. Szklarczyk, D., Gable, A. L., Lyon, D., Junge, A., Wyder, S., Huerta-Cepas, J., Simonovic, M., Doncheva, N. T., Morris, J. H., Bork, P., Jensen, L. J., & Mering, C. V. (2019). STRING v11: Protein–protein association networks with increased coverage, supporting functional discovery in genome-wide experimental datasets. *Nucleic Acids Research*, *47*(D1), D607–D613. https://doi.org/10.1093/nar/gky1131
22. Ling, Y., Galusca, B., Martin, F.-P., Bartova, S., Carayol, J., Moco, S., Epelbaum, J., Grouselle, D., Boirie, Y., Montaurier, C., Cuenco, J., Minnion, J. S., Thomas, T., Mure, S., Hager, J., Estour, B., Gheldof, N., & Germain, N. (2020). Resistance to lean mass gain in constitutional thinness in free-living conditions is not overpassed by overfeeding. *Journal of Cachexia, Sarcopenia and Muscle*, *11*(5), 1187–1199. https://doi.org/10.1002/jcsm.12572
23. Haywood, N. J., Slater, T. A., Matthews, C. J., & Wheatcroft, S. B. (2019). The insulin like growth factor and binding protein family: Novel therapeutic targets in obesity & diabetes. *Molecular Metabolism*, *19*, 86–96.
24. Zhu, Y., Mendola, P., Albert, P. S., Bao, W., Hinkle, S. N., Tsai, M. Y., & Zhang, C. (2016). Insulin-like growth factor axis and gestational diabetes mellitus: A longitudinal study in a multiracial cohort. *Diabetes*, *65*, 3495–3504. https://doi.org/10.2337/db16-0514
25. Ahmed, R. L., Thomas, W., & Schmitz, K. H. (2007). Interactions between insulin, body fat, and insulin-like growth factor axis proteins. *Cancer Epidemiology Biomarkers and Prevention*, *16*(3), 593–597. https://doi.org/10.1158/1055-9965.EPI-06-0775
26. Hu, D., Pawlikowska, L., Kanaya, A., Hsueh, W. C., Colbert, L., Newman, A. B., Satterfield, S., Rosen, C., Cummings, S. R., Harris, T. B., & Ziv, E. (2009). Serum insulin-like growth factor-1 binding proteins 1 and 2 and mortality in older adults: The health, aging, and body composition study. *Journal of the American Geriatrics Society*, *57*(7), 1213–1218. https://doi.org/10.1111/j.1532-5415.2009.02318.x
27. Aronson, D., Bartha, P., Zinder, O., Kerner, A., Markiewicz, W., Avizohar, O., Brook, G. J., & Levy, Y. (2004). Obesity is the major determinant of elevated C-reactive protein in subjects with the metabolic syndrome. *International Journal of Obesity*, *28*, 674–679. https://doi.org/10.1038/sj.ijo.0802609
28. Silha, J. V., Krsek, M., Sucharda, P., & Murphy, L. J. (2005). Angiogenic factors are elevated in overweight and obese individuals. *International Journal of Obesity*, *29*(11), 1308–1314. https://doi.org/10.1038/sj.ijo.0802987
29. Carayol, J., Chabert, C., Di Cara, A., Armenise, C., Lefebvre, G., Langin, D., Viguerie, N., Metairon, S., Saris, W. H. M., Astrup, A., Descombes, P., Valsesia, A., & Hager, J. (2017). Protein quantitative trait locus study in obesity during weight-loss identifies a leptin regulator. *Nature Communication*, *8*, 2084. https://doi.org/10.1038/s41467-017-02182-z
30. Strong, A. L., Semon, J. A., Strong, T. A., Santoke, T. T., Zhang, S., Mcferrin, H. E., Gimble, J. M., & Bunnell, B. A. (2012). Obesity-associated dysregulation of calpastatin and MMP-15 in adipose-derived stromal cells results in their enhanced invasion. *Stem Cells*, *30*(12), 2774–2783. https://doi.org/10.1002/stem.1229
31. Mullur, R., Liu, Y. Y., & Brent, G. A. (2014). Thyroid hormone regulation of metabolism. *Physiological Reviews*, *94*(2), 355–382. https://doi.org/10.1152/physrev.00030.2013
32. Mao, J., & Teng, W. (2013). The relationship between selenoprotein P and glucose metabolism in experimental studies. *Nutrients*, *5*(6), 1937–1948. https://doi.org/10.3390/nu5061937
33. Moreno, O., Cominetti, O., Núñez Galindo, A., Irincheeva, I., Corthésy, J., Astrup, A., Saris, W. H. M., Hager, J., Kussmann, M., & Dayon, L. (2018). The differential plasma proteome of obese and overweight individuals undergoing a nutritional weight loss and maintenance intervention. *Proteomics Clinical Applications*, *12*(1), 1600150. https://doi.org/10.1002/prca.201600150
34. Calcaterra, V., De Amici, M., Leonard, M. M., De Silvestri, A., Pelizzo, G., Buttari, N., Michev, A., Leggio, M., Larizza, D., & Cena, H. (2018). Serum calprotectin level in children: Marker of obesity and its metabolic complications. *Annals of Nutrition & Metabolism*, *73*, 177–183. https://doi.org/10.1159/000492579
35. Schmid, A., Petry, N., Walther, B., Bütikofer, U., Luginbühl, W., Gille, D., Chollet, M., Mcternan, P. G., Gijs, M. A. M., Vionnet, N., Pralong, F.

P., Laederach, K., & Vergères, G. (2015). Inflammatory and metabolic responses to high-fat meals with and without dairy products in men. *British Journal of Nutrition*, *113*(12), 1853–1861. https://doi.org/10.1017/S0007114515000677
36. Duan, Y., Zeng, L., Zheng, C., Song, B., Li, F., Kong, X., & Xu, K. (2018). Inflammatory links between high fat diets and diseases. *Frontiers in Immunology*, *9*, 2649. https://doi.org/10.3389/fimmu.2018.02649
37. Ukkola, O., Sun, G., & Bouchard, C. (2001). Insulin-like growth factor 2 (IGF2) and IGF-binding protein 1 (IGFBP1) gene variants are associated with overfeeding-induced metabolic changes. *Diabetologia*, *44*, 2231–2236. https://doi.org/10.1007/s001250100034
38. Atshaves, B. P., Martin, G. G., Hostetler, H. A., Mcintosh, A. L., Kier, A. B., & Schroeder, F. (2010). Liver fatty acid-binding protein and obesity. *Journal of Nutritional Biochemistry*, *21*(11), 1015–1032. https://doi.org/10.1016/j.jnutbio.2010.01.005
39. Jung, S. Y., Hursting, S. D., Guindani, M., Vitolins, M. Z., Paskett, E., & Chang, S. (2014). Bioavailable insulin-like growth factor-I inversely related to weight gain in postmenopausal women regardless of exogenous estrogen. *Cancer Epidemiology and Prevention Biomarkers*, *23*(3), 534–544. https://doi.org/10.1158/1055-9965.EPI-13-1053
40. Park, J., Rho, H. K., Kim, K. H., Choe, S. S., Lee, Y. S., & Kim, J. B. (2005). Overexpression of glucose-6-phosphate dehydrogenase is associated with lipid dysregulation and insulin resistance in obesity. *Molecular and Cellular Biology*, *25*(12), 5146–5157.
41. Geyer, P. E., Wewer Albrechtsen, N. J., Tyanova, S., Grassl, N., Iepsen, E. W., Lundgren, J., Madsbad, S., Holst, J. J., Torekov, S. S., & Mann, M. (2016). Proteomics reveals the effects of sustained weight loss on the human plasma proteome. *Molecular Systems Biology*, *12*, 901. https://doi.org/10.15252/msb.20167357
42. Harney, D. J., Hutchison, A. T., Hatchwell, L., Humphrey, S. J., James, D. E., Hocking, S., Heilbronn, L. K., & Larance, M. (2019). Proteomic analysis of human plasma during intermittent fasting. *Journal of Proteome Research*, *18*(5), 2228–2240.
43. Fernández-Cao, J. C., Arija, V., Aranda, N., Basora, J., Diez-Espino, J., Estruch, R., Fitó, M., Corella, D., & Salas-Salvadó, J. (2017). Soluble transferrin receptor and risk of type 2 diabetes in the obese and nonobese. *European Journal of Clinical Investigation*, *47*, 221–230.
44. Germain, N., Galusca, B., Caron-Dorval, D., Martin, J. F., Pujos-Guillot, E., Boirie, Y., Khalfallah, Y., Ling, Y., Minnion, J. S., Bloom, S. R., Epelbaum, J., & Estour, B. (2014). Specific appetite, energetic and metabolomics responses to fat overfeeding in resistant-to-bodyweight-gain constitutional thinness. *Nutrition & Diabetes*, *4*, e126.
45. Galusca, B., Verney, J., Meugnier, E., Ling, Y., Edouard, P., Feasson, L., Ravelojaona, M., Vidal, H., Estour, B., & Germain, N. (2018). Reduced fibre size, capillary supply and mitochondrial activity in constitutional thinness' skeletal muscle. *Acta Physiologica (Oxf)*, *224*(3), e13097.

SUPPORTING INFORMATION

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

How to cite this article: Cominetti, O., Galindo, A. N., Corthésy, J., Carayol, J., Germain, N., Galusca, B., Estour, B., Hager, J., Gheldof, N., & Dayon, L. (2022). Proteomics reveals unique plasma signatures in constitutional thinness. *Prot. Clin. Appl.*, *16*, e2100114. https://doi.org/10.1002/prca.202100114