

## ARTICLE

# Ciliary neurotrophic factor (CNTF) genotype and body composition

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Exogenous ciliary neurotrophic factor (CNTF) administration causes significant weight loss in both humans and animal models, but the effects of endogenous CNTF and the CNTF null allele on body composition are not fully understood. A recent study in a European cohort demonstrated a significantly higher body weight and body mass index (BMI) in older males homozygous for the CNTF null allele (A/A genotype). We sought to replicate these findings in three cohorts: the Baltimore Longitudinal Study on Aging (BLSA) consisting of 422 adult men and women (19–90 years); the Study of Osteoporotic Risk in Men (STORM) consisting of 333 older men (50–84 years); and a third sample obtained by combining older males aged 59–73 years from the BLSA and STORM cohorts ( $n=286$ ). In contrast to the European study, we were unable to detect a significant association between CNTF genotype and body weight in the BLSA ( $P=0.49$ ), the STORM ( $P=0.28$ ), or the combined samples ( $P=0.72$ ). There was also no significant association observed between CNTF genotype and BMI in the BLSA ( $P=0.59$ ), the STORM ( $P=0.34$ ) or the combined ( $P=0.56$ ) samples. In addition, we were unable to detect a significant association between CNTF genotype and total body fat ( $P=0.95$ ) or fat-free mass ( $P=0.86$ ) in the BLSA cohort. Our results do not support an effect of the CNTF null allele on body composition, contrary to previous findings.

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## Introduction

Ciliary neurotrophic factor (CNTF), a neurotrophic cytokine for motor neurons, caused significant weight loss in subjects with multiple sclerosis and amyotrophic lateral sclerosis.<sup>1</sup> Subsequent studies indicated that CNTF may influence body weight by activating leptin-like intracellular signaling pathways (Janus kinases and signal

transducers and activators of transcription 3 (JAK/STAT3)) in hypothalamic nuclei that regulate appetite and body weight.<sup>2,3</sup> CNTF may also regulate appetite and body weight by a mechanism independent of leptin and involving STAT3 activation in the arcuate nuclei.<sup>4</sup> However, it remains unclear if endogenous CNTF has a similar influence on body weight and composition. The expression of endogenous CNTF is restricted to schwann cells and astrocytes,<sup>5,6</sup> and CNTF is not secreted by the classical pathway involving the endoplasmic reticulum and the golgi complex,<sup>7</sup> and consequently does not circulate in the peripheral circulation.<sup>7,8</sup>

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Takahashi *et al*<sup>9</sup> described a G-to-A substitution at position -6 of the second exon of the CNTF gene, which leads to a frameshift mutation and a truncated, biologically inactive protein. This mutation results in the total absence of functional CNTF protein in null homozygotes (A/A).<sup>9</sup> Most evidence suggests that the CNTF null mutation does not influence body weight or composition. For example, CNTF knockout mice are not obese, and there is no association between CNTF genotype and morbid obesity in children and adolescents.<sup>10,11</sup> Recently, O'Dell *et al*<sup>12</sup> reported significantly higher body weight and body mass index (BMI) for males homozygous for the null mutation, with no significant effect in females, but these results have not been replicated. They hypothesized that, since exogenous CNTF administration resulted in weight loss, the absence of functional CNTF in the (A/A) homozygotes would similarly result in decreased initiation of anorectic pathways, with a consequent increase in body weight and BMI. Given the uncertainty surrounding endogenous CNTF and body composition in particular, and the influence of specific genes on obesity and body composition in general, we sought to replicate the findings of O'Dell *et al*<sup>12</sup> by analyzing CNTF genotype in three samples of Caucasian adults from the United States.

## Materials and methods

### Subjects

The BLSA cohort consisted of 422 volunteers (225 men and 197 women) aged 19–90 years. Details of the BLSA study are outlined elsewhere.<sup>13</sup> All BLSA subjects received a complete medical history and physical examination, and subjects with clinical cardiovascular or musculoskeletal disorders that could be adversely affected by exercise testing were excluded. The detailed exclusionary criteria are outlined elsewhere.<sup>14,15</sup> The STORM cohort consisted of a sample of 333 Caucasian males aged 50–84 years recruited from population-based listings.<sup>16</sup> Men who were unable to walk without the assistance of another person or had undergone a bilateral hip replacement were ineligible to participate in STORM.

All subjects gave their written informed consent prior to participation and received a complete medical history and physical examination. The experimental protocols were approved by the Institutional Review Boards (IRBs) for Human Subjects at Johns Hopkins Bayview Medical Center (Baltimore, MD, USA) and at the University of Pittsburgh. The protocols related to the analysis of genetic data were approved by IRBs at the University of Maryland (College Park, MD, USA) and the University of Pittsburgh (Pittsburgh, PA, USA).

### CNTF genotype

DNA was isolated from EDTA anticoagulated whole blood using methods previously described.<sup>17</sup> The G-to-A CNTF

null mutation was identified by a *Hae*III digestion of the polymerase chain reaction (PCR)-amplified genomic DNA, as previously described,<sup>9</sup> and the method was verified by direct DNA sequencing of a random sample of subjects. Subjects were characterized as exhibiting the G/G, G/A or A/A genotype.

### Body composition

For volunteers from the BLSA cohort, body mass and height were measured for each subject to the nearest 0.1 kg and 0.5 cm, respectively, using a medical beam scale, and BMI was calculated ( $\text{kg}/\text{m}^2$ ). Assessment for total body fat and fat-free mass was also assessed by dual-energy X-ray absorptiometry (DXA) in 374 volunteers, using previously described methods.<sup>15</sup> Volunteers from the STORM cohort had their body weight measured to the nearest 0.1 kg on a calibrated balance beam scale. Height was measured to the nearest 0.1 cm after removal of shoes, at the peak of inhalation, using a wall-mounted Harpenden stadiometer (Holtain, Dyved, UK). The average of two height measurements was used and BMI was calculated ( $\text{kg}/\text{m}^2$ ). No measures of fat mass were available in the STORM cohort.

### Physical activity

Physical activity was estimated for each of the BLSA participants using self-reported time spent in 97 activities, as has been previously reported.<sup>18,19</sup> Physical activity was quantified into MET minutes, based on the metabolic equivalent of each particular activity and the time spent in that activity, normalized to 24 h.

STORM participants also completed a self-administered questionnaire, which was reviewed with each participant in the clinic by a trained interviewer. In addition to medical history, physical activity was measured using a modified Paffenbarger Scale,<sup>20</sup> in which subjects reported the frequency and duration of their participation per week during the past year in 33 different physical activities. The activities were assigned energy expenditures according to previously reported methods.<sup>21</sup> Total physical activity, expressed in kilocalories expended per day, was calculated by adding kilocalories expended in the 33 recreational activities.

### Statistics

$\chi^2$  statistics were used to assess genotype frequencies and the deviation from Hardy–Weinberg equilibrium. Analysis of variance (ANOVA) was used to assess the physical characteristics by CNTF genotype. Body mass, BMI, body fat, and fat-free mass (BLSA only) were also analyzed using analysis of covariance (ANCOVA). For these analyses, we adjusted for gender (BLSA only) age, physical activity, and height (except for BMI). To more directly replicate the findings of O'Dell *et al*,<sup>12</sup> we performed these similar analyses on the combined sample, which included all the Caucasian males aged 59–73 years in the BLSA and the

STORM cohorts, to match the Hertfordshire cohort of O'Dell *et al* as closely as possible.<sup>12</sup> We had 80% statistical power at  $P=0.05$  to identify a difference of 2.5 kg/m<sup>2</sup> in BMI between the G/G and A/A groups in this combined cohort. Data are reported as least squares means  $\pm$  standard error of the mean (SE). Statistical significance was accepted at  $P<0.05$ .

## Results

### BLSA cohort

Of the 422 total subjects in the BLSA cohort, 324 exhibited the G/G genotype (76.7%), 89 exhibited the G/A genotype (21.1%), and nine exhibited the A/A genotype (2.13%). These genotype frequencies were not different from those predicted by Hardy–Weinberg equilibrium ( $P=0.33$ ). The A allele frequency was 12.7%. No significant genotype differences were observed with regard to subject characteristics, as shown in Table 1.

No significant differences were observed among the three CNTF genotype groups for total body weight ( $P=0.49$ ), BMI ( $P=0.59$ ) or height ( $P=0.73$ ) in either men or women (Table 1). There was also no significant interaction between genotype and age in any analysis. The BLSA cohort had data on fat mass as well as fat-free mass, and neither fat ( $P=0.95$ ) nor fat-free mass ( $P=0.86$ ) were significantly associated with CNTF genotype (Table 1).

### STORM cohort

Of the 333 total subjects in the STORM cohort, 236 exhibited the G/G genotype (70.9%), 87 exhibited the G/A genotype (26.1%), and 10 exhibited the A/A genotype (3.0%). These genotype frequencies were not different from those predicted by Hardy–Weinberg equilibrium ( $P=0.57$ ). The A allele frequency was 16.1%. No significant genotype differences were observed with regard to subject characteristics (Table 2). No significant differences were observed among the three genotype groups for total body weight ( $P=0.28$ ) or BMI ( $P=0.34$ ; Table 2).

### Combined sample

Of the 286 total Caucasian men aged 59–73 years in the combined BLSA and STORM cohorts, 207 exhibited the G/G genotype (72.4%), 72 exhibited the G/A genotype (25.2%), and seven exhibited the A/A genotype (2.4%). These genotype frequencies were not different from those predicted by Hardy–Weinberg equilibrium ( $P=0.80$ ). The A allele frequency was 15.0%. Similar to the analyses in the individual cohorts, no significant genotype differences were observed with regard to subject characteristics, and no significant differences were observed for total body weight ( $P=0.72$ ) or BMI ( $P=0.56$ ; Table 3). Similar nonsignificant results were observed in a larger ( $n=452$ ) combined group of men from these cohorts aged 50–93 years (data not shown).

**Table 1** Subject characteristics, weight, body mass index (BMI), fat mass, and physical activity by CNTF genotype in 422 Caucasian adult men and women from the BLSA cohort

	G/G genotype		G/A genotype		A/A genotype		P-value
	Men	Women	Men	Women	Men	Women	
Subjects (n)	168	156	51	38	6	3	
Age (year)	58.5 $\pm$ 1.2	49.3 $\pm$ 1.2	55.3 $\pm$ 2.1	46.5 $\pm$ 2.4	61.9 $\pm$ 5.8	39.7 $\pm$ 8.9	0.22
Height (cm)	178.2 $\pm$ 0.5	164.2 $\pm$ 0.5	177.7 $\pm$ 0.8	163.6 $\pm$ 1.0	177.4 $\pm$ 2.5	163.6 $\pm$ 3.5	0.73
Weight (kg)	86.9 $\pm$ 1.1	68.7 $\pm$ 1.2	87.1 $\pm$ 2.0	65.8 $\pm$ 2.4	80.4 $\pm$ 5.9	65.2 $\pm$ 8.3	0.49
BMI (kg/m <sup>2</sup> )	27.3 $\pm$ 0.4	25.4 $\pm$ 0.4	27.6 $\pm$ 0.6	24.6 $\pm$ 0.7	25.5 $\pm$ 1.8	24.2 $\pm$ 2.5	0.59
Fat mass (kg) <sup>a</sup>	24.2 $\pm$ 0.8	26.2 $\pm$ 0.8	24.5 $\pm$ 1.4	25.5 $\pm$ 1.6	23.9 $\pm$ 3.6	24.5 $\pm$ 5.4	0.94
Physical activity (MET-min/24 h)	2222 $\pm$ 34	2350 $\pm$ 35	2270 $\pm$ 61	2413 $\pm$ 71	2277 $\pm$ 167	2427 $\pm$ 255	0.55

Data are least squares means  $\pm$  SE.

<sup>a</sup>Data are available for 374 volunteers.

**Table 2** Subject characteristics, weight, body mass index (BMI) by CNTF genotype in 333 adult men from the STORM cohort

	G/G genotype	G/A genotype	A/A genotype	P-value
Subjects (n)	236	87	10	
Age (year)	65.9 $\pm$ 0.5	66.9 $\pm$ 0.8	64.1 $\pm$ 2.3	0.38
Height (cm)	174 $\pm$ 0.4	174 $\pm$ 0.7	173 $\pm$ 2.0	0.54
Weight (kg)	82.8 $\pm$ 0.8	85.1 $\pm$ 1.3	85.4 $\pm$ 3.9	0.28
BMI (kg/m <sup>2</sup> )	27.4 $\pm$ 0.2	28.0 $\pm$ 0.4	28.7 $\pm$ 1.2	0.34
Physical activity (kcal/day)	314 $\pm$ 18	238 $\pm$ 29	147 $\pm$ 86	0.02

Data are least squares means  $\pm$  SE.

**Table 3** Subject characteristics, weight, body mass index (BMI) by CNTF genotype in 286 Caucasian adult males (59–73 years) from the BLSA and STORM cohorts

	G/G genotype	G/A genotype	A/A genotype	P-value
Subjects (n)	207	72	7	
Age (year)	66.4±0.3	66.7±0.5	66.9±1.6	0.88
Height (cm)	175±0.4	175±0.7	173±2.4	0.68
Weight (kg)	84.3±0.9	84.9±1.5	88.1±4.8	0.72
BMI (kg/m <sup>2</sup> )	27.7±0.3	27.7±0.4	29.2±1.4	0.56

Data are least squares means±SE.

## Discussion

Exogenous CNTF administration causes significant weight reduction in humans and animal models by both leptin-like and leptin-independent mechanisms.<sup>3,22</sup> Ettinger *et al*<sup>22</sup> recently reported that obese individuals who were treated with a genetically engineered recombinant human variant CNTF (rhvCNTF; Axokine) for 12 weeks demonstrated significantly more weight loss than those treated with a placebo. However, whether endogenous CNTF plays a physiologic role in weight control is questionable, as most studies show that both mice and humans lacking CNTF are not obese.<sup>10,11</sup> While CNTF is present in Schwann cells and astrocytes,<sup>5,6</sup> it is not released into the peripheral circulation under normal conditions<sup>7,8</sup> and, since the predicted human CNTF protein contains no signal peptide, it raises the question of how CNTF would be released. It is possible that CNTF is only released locally under pathological conditions, such as during nerve injury to promote neuronal regeneration. Since CNTF is not secreted by the nerve cells into the circulation, it seems unlikely that CNTF plays a significant role in the physiological regulation of body composition in humans. We should note, however, that exogenous administration of CNTF may have a different mechanism of action than that of endogenous CNTF. For example, Dittrich *et al*<sup>23</sup> observed uptake of exogenous CNTF into the liver, and other non-neuronal cells are responsive to CNTF.<sup>7</sup> O'Dell *et al*<sup>12</sup> hypothesized and found that the absence of endogenous CNTF as a result of homozygosity for the CNTF null allele results in a diminished initiation of anorectic pathways, with a consequent increase in body mass in those with the A/A genotype. O'Dell *et al*<sup>12</sup> reported 10 kg higher weight and 3 kg/m<sup>2</sup> higher BMI in nine older Caucasian men with the A/A genotype compared to those with the G/G genotype ( $n=407$ ). Our study sought to replicate these findings by examining two independent cohorts of Caucasian adults.

Neither of the two cohorts that we examined demonstrated a significant relationship between CNTF genotype and body weight, BMI, or body composition. To more closely replicate the findings of O'Dell *et al*,<sup>12</sup> we also performed a third combined analysis on a subgroup of our

subjects that matched their cohort on age, and we similarly observed no association between CNTF genotype and body weight and BMI. While there is a documented association between CNTF genotype and muscular strength, even though the basis for the function of the CNTF null allele in muscular strength is unclear,<sup>24</sup> we speculate that the lack of association with body composition in the current study may be as a result of a localized action of CNTF at the neuromuscular junction rather than a systemic or CNS-localized action. Thus, the polymorphism could impact CNTF gene expression only locally, affecting motor unit function and thus strength, and not influencing body composition.

Hoffmann *et al*<sup>25</sup> found no association between the CNTF null allele genotype and age of onset, progression, or severity of multiple sclerosis. They concluded that the requirement for CNTF in myelogenesis or cell survival may be bypassed by a second yet-to-be-identified ligand, and this second ligand might compensate for the absence of functional CNTF in A/A homozygotes.<sup>25</sup> Indeed, the CNTF receptor (CNTFR) acts as a receptor for cardiotrophin-like cytokine (CLC), which has been shown to support the survival of motor and sympathetic neurons *in vitro*,<sup>26</sup> but little else is known about CLC function. Roth *et al*<sup>27</sup> have described a polymorphism in the CNTFR gene that is associated with fat-free mass in both men and women, but no association was observed with fat mass.

A small but statistically significant difference was noted among genotype groups for physical activity values in the STORM cohort. The nature of this association is unclear; however, physical activity was not a significant contributor to any ANCOVA model in either the BLSA or STORM analyses for BMI and other variables. While physical activity was a covariate in both the STORM and BLSA cohorts, the measures used differed enough that physical activity was excluded as a covariate in the combined analysis.

We conclude that, contrary to the findings of O'Dell *et al*,<sup>12</sup> the null A allele of the CNTF gene does not influence body composition in Caucasian adults. The basis of this difference between these studies is uncertain, but the issue of population stratification may be relevant.

Although all the three groups (ie, BLSA, STORM, and Hertfordshire) were Caucasian of European ancestry, greater homogeneity in the O'Dell *et al*<sup>12</sup> Hertfordshire sample could be argued given the recruitment strategies of those subjects. In both studies, the sample size for CNTF A/A individuals is lower than 15, which limits the ability to control for other genetic and environmental influences on body composition. Moreover, both studies can only be descriptive, as the mechanism by which endogenous CNTF could regulate body fat mass is still not understood.

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