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A Functional *GNAS* Promoter Polymorphism is Associated with Altered Weight Loss during Short-term Fasting

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Abstract
In mice, heterozygous knockout of the stimulatory G protein Gαs results in obesity which suggests a key role of Gαs in body weight regulation. We have recently identified a functional G(-1211)A promoter polymorphism in the human *GNAS* gene encoding Gαs, the GG genotype being associated with increased promoter activity and lipolysis *in vitro* and increased weight loss capacity *in vivo*. The present study aimed to independently confirm these results. We genotyped 87 subjects who underwent a 7-day modified fast for the *GNAS* polymorphism and recorded weight, hunger, and mood. While both mood and hunger were not dependent on genotype, *GNAS* genotypes were significantly associated with weight loss (GG: -5.0 ± 1.5 kg, n=28; AG: -4.2 ± 1.1 kg, n = 50; AA: -3.2 ± 1.2, n = 9; p = 0.0003). The present study reconfirms our earlier reported findings and suggests that *GNAS* genotypes also influence weight loss during short-term fasting.

Fasting is practised in different cultures around the world, mostly periodically in a traditional religious or spiritual context. In its modern approach, fasting consists of controlled underfeeding for 5–20 days with a large intake of fluids (tea, water, juice) and a low caloric intake of 200–300 kcal/day (modified fasting) [1]. The medical use of fasting has focused on the treatment of rheumatoid and pain syndromes [2]. However, fasting was also shown to lead to mood enhancement and it improves the quality of sleep and daytime performance in non-obese subjects [3]. The well-known metabolic reactions to fasting are accompanied by a broad range of neuroendocrine changes. Activation of the hypothalamic-pituitary-adrenal axis is reflected by increased concentrations of adrenaline, noradrenaline, and cortisol within 48 h after the onset of fasting [4]. Additional adaptive hormonal responses to fasting include a decrease in serum insulin and leptin and an increase in growth hormone [5]. Fasting is also accompanied by weight loss resulting from an increased activation of the sympathetic nervous system and increased lipolysis [6]. In adipocytes, an increase in cAMP is associated with lipolysis which is supposed to be Gαs dependent [7;8] and the pivotal role of the gene *GNAS* which encodes the G protein subunit Gαs, in body weight control is underscored by phenotypic changes in animals in which different *gnas* gene products are knocked out [9]. We have recently shown that a functional G(-1211)A polymorphism in the *GNAS* promoter affects binding of the lipolysis-associated transcription factor USF1, lipolysis, and weight regulation in men [10]. We, therefore, examined a potential association between genotypes of the G(-1211)A polymorphism, weight loss, and mood change in 87 consecutive patients (mean age 50.1 ± 13.7 years; 24 male/ 63 female) who were admitted to an Internal Medicine ward specialised in nutritional therapies to participate in an seven-day modified fast. All patients were admitted for the treatment of the metabolic syndrome and/or chronic pain syndromes of the locomotor system and/or metabolic syndrome. Patients with severe obesity (BMI> 40kg/m^2), binge-eating disorder, psychiatric disease, or medication with antidepressant or psychotropic medications were excluded. Fasting was prescribed to support subsequent intensified lifestyle modification and consisted of seven days of caloric restriction to 300 kcal total energy intake/day as described elsewhere [11]. Fasting was preceded by two low-calorie diet days (1000 kcal intake/day) and followed by three days with stepwise reintroduction of solid foods. To exclude patients with manifest depression the hospital-anxiety depression scale (HADS) was used. Body weight was measured daily at 07:30 h a.m. on a calibrated scale in light clothing without shoes. Mood was assessed daily at 18:00 h by means of a self-rating 10-point numeric visual analogue scale (VAS) with a value of 10 indicating best and 0 indicating worst mood. Patients were carefully instructed before first self-ratings on the correct use of the visual analogue scale and asked to place the mark so that it best indicated their mood state on the respective day. Perceived hunger was recorded by a self-rating 10-point numeric scale with 0 indicating "not hungry at all", and 10 indicating "extremely hungry". Data collection was performed by personnel blinded for genotypes. Genotyping was performed as described [10], with the genotyping laboratory being blinded for the clinical results. Data are presented as means ± S.D. until stated otherwise. Baseline values were compared by univariate analysis of variance (ANOVA) for continu-

Table 1. Demographic characteristic of the study group.

	AA	AG	GG	All	P
N	9	50	28	87	
Sex [male\female]	2/7	15/35	9/19	24/63	n.s.
Age [years]	43.8 ± 13.5	52.0 ± 13.9	49.4 ± 13.2	50.1 ± 13.7	n.s.
Weight [kg]	78.3 ± 18.4	80.1 ± 15.2	83.4 ± 20.7	81.2 ± 17.7	n.s.
BMI [kg/m^2]	28.1 ± 6.0	28.3 ± 4.8	28.6 ± 6.8	28.4 ± 5.7	n.s.
Hunger [VAS]	4.25 ± 1.6	4.38 ± 1.31	4.07 ± 1.68	4.26 ± 1.47	n.s.
Mood [VAS]	6.04 ± 2.34	6.36 ± 1.94	6.26 ± 2.12	6.28 ± 2.04	n.s.

Data are presented as mean ± S.D. BMI, VAS, visual analogue scale (0=worst, 10=best).

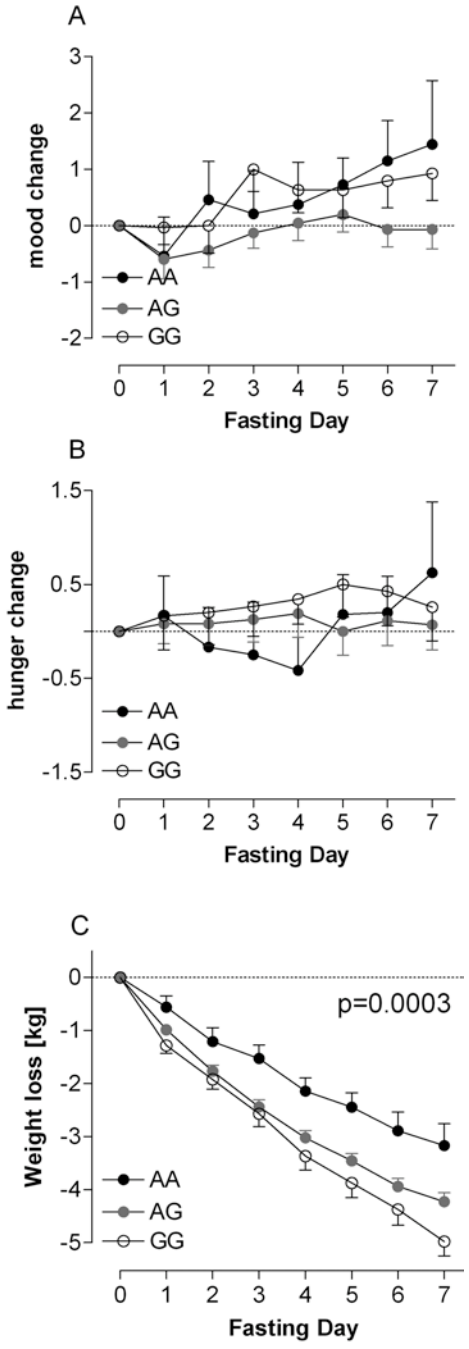

Fig. 1. Mean change (±SEM) of mood (A), hunger (B), and weight (C) during extended fasting for *GNAS* genotypes. p-value is given using linear ANOVA.

ous variables, and by chi-square-test test for categorical variables. Endpoint measures of weight loss were analyzed by ANOVA and analysis of covariance (ANCOVA) with baseline values as covariates as indicated. Changes from baseline of mood and hunger were analyzed by ANCOVA for repeated measures with baseline values as covariates. Differences were regarded significant at a *P*-value <0.05. The study was approved by our ethics committee and all patients gave their written informed consent.

Genotype distribution was compatible with the Hardy-Weinberg equilibrium and demographic characteristics are given in Table1. Age, gender, weight, and baseline ratings of mood and perceived hunger were not significantly different between genotypes. We observed no genotype-dependent differences in mood and hunger changes (Fig. 1A+B). However, weight loss was dependent on *GNAS* genotypes. GG genotypes lost significantly more weight compared to AA and AG genotypes (GG: -5.0 ± 1.5 kg, n = 28; AG: -4.2 ± 1.1 kg, n = 50; AA: -3.2 ± 1.2, n = 9; p = 0.0003; Fig. 1C) demonstrating a gene-dose effect. This effect remained statistically significant after adjusting for baseline weight as covariate (p = 0.006).

The present data support the hypothesis that fasting-induced weight loss is significantly associated with genotypes of the G(-1211)A polymorphism. The results are in line with our previous finding demonstrating that GG genotypes lost significantly more weight compared to A allele carriers in a 54 week-lasting structured weight loss program. The G allele results in stronger binding of the transcription factor USF1, which controls the expression of genes involved in glucose and lipid metabolism [10]. In adipose tissue, USF1 influences the glucose-regulated expression of hormone-sensitive lipase [12], the key enzyme for mobilization of fatty acid from triglycerides. Complete fasting is known to cause profound hormonal changes and upregulation of transcription factors [13] including upregulation of USF1 [12]. A more effective activation of the (-1211)G allele following these hormonal changes was shown to result in increased Gαs expression and lipolysis [10] and could, therefore, explain the different genotype-dependent weight loss capacities on fasting. This is also in accordance with previous studies showing that Gαs deficiency results in obesity and impaired lipolytic response to catecholamines [14, 15]. Although genetic variance could at least in part explain the large interindividual varia-

tion in the action of catecholamines on lipolysis in apparently healthy subjects [16], we are well aware of alternative mechanisms which may account for the associations described here. Nevertheless, we believe that replication of a genetic association study in an independent population is essential in the field of pharmacogenetic research. Therefore, our study may substantiate the role of the G(-1211)A polymorphism in body weight regulation.

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