Elevated plasma ghrelin levels in Prader–Willi syndrome

To the editor—Recent advances have begun to clarify the pathogenesis of genetic obesity in humans. Mutations that affect signaling by leptin and melanocortins were hypothesized to contribute to human obesity based on their important roles in body-weight regulation in rodent models, and compelling support for this hypothesis now exists[1–3]. Ghrelin is a novel enteric hormone[4] that increases food intake, body weight and growth-hormone (GH) secretion as potently as any known peptide[4–7]. As the only known circulating orexigen, ghrelin is implicated in meal-time hunger[8] and body-weight regulation in rodents[5,6] and humans[9,10]. However, previous studies have not investigated the hypothesis that ghrelin hypersecretion might contribute to genetic obesity.

Prader–Willi syndrome (PWS) is the most common form of human syndromic obesity. It is characterized by severe hyperphagia, GH deficiency, hypogonadism, neonatal hypotonia, dysmorphic features and cognitive impairment[11]. Although the genetic basis of PWS involves imprinting disorders of several genes on chromosome 15, mediators of the phenotype are unknown[12]. Because ghrelin affects appetite as well as GH secretion, and both are abnormal in PWS, we hypothesized that this condition might involve ghrelin dysregulation. To investigate whether ghrelin might have a role in the pathogenesis of PWS or other forms of genetic obesity, we measured plasma ghrelin levels in humans with PWS, leptin receptor (LepR) mutations[2], melanocortin-4 receptor (Mc4r) mutations[3,13] and appropriate controls (Table 1).

Table 1 Subject characteristics

Group	Number	Female:Male	Age (y)	BMI (kg/m^2)
PWS	18	8:10	24.6 ± 5.0	33.9 ± 9.0
Obese controls[a]	14	10:4	41.4 ± 7.7	36.1 ± 3.4
Lean controls[b]	16	9:7	42.8 ± 11.0	25.1 ± 3.5
LepR mutants[c]	2	2:0	16.0 ± 4.2	68.6 ± 4.2
LepR controls[d]	7	4:3	27.0 ± 15.6	27.4 ± 5.6
Mc4r mutants[e]	19	10:9	29.8 ± 15.7	34.8 ± 10.3
Mc4r controls[f]	22	15:7	33.9 ± 15.0	31.8 ± 12.0

[a], BMI = 30 kg/m^2, genetically uncharacterized obesity. [b], BMI < 30 kg/m^2. [c], Exon/intron 16 G/A substitution; truncated receptor lacks transmembrane and intracellular domains (ref. 2). [d], First-degree relatives of subjects with LepR mutations (5 heterozygotes and 2 wild type). [e], Each subject has one of the following heterozygous Mc4r mutations (refs. 3,13 and data not shown): InsG47-48 frameshift (inactivates), Ile102Ser (inactivates), Ser295Pro (partially inactivates), Ile170Val (inactivates), Val50Met (partially inactivates), Ile251Leu (polymorphism), Ser58Cys (inactivates), Ala154Asp (partially inactivates), Val103Ile (polymorphism) or Leu250Gln (constitutively activates *in vitro*, but associated with obesity). [f], Unaffected first-degree relatives of all but 4 Mc4r mutants, for whom no family controls were available. For these 4, unrelated controls matched for age, sex and BMI were used. Values shown are mean ± s.d.

Overnight fasting plasma ghrelin levels were 4.5-fold higher in PWS subjects (1542 ± 271 pg/ml) than in equally obese controls (344 ± 45 pg/ml, $P < 0.001$), and 2.5-fold higher than in lean controls (608 ± 68 pg/ml, $P = 0.003$) (Fig. 1*a*). The mechanism underlying this increase is unlikely to reflect mutation of the genes encoding ghrelin or its receptor, as these are not contained within the locus responsible for PWS. However, several affected genes in that region encode factors that could indirectly affect ghrelin expression[12]. High ghrelin levels in PWS are unlikely to arise from deficient negative feedback by GH, as GH does not appear to regulate circulating ghrelin[14].

Ghrelin secretion is reported to be inhibited by leptin[15], and PWS individuals may be leptin-resistant, in view of their hyperphagia despite high leptin levels[16]. However, hyperghrelinemia is unlikely to arise from leptin resistance, as ghrelin levels are not elevated in obese subjects homozygous for an inactivating LepR mutation (Fig. 1*a*). This finding suggests that leptin signaling is not an important determinant of human ghrelin levels. Similarly, abnormalities in Mc4r, a critical central nervous system target of leptin action, are unlikely to explain increased ghrelin levels in PWS. We found low or normal ghrelin levels in subjects with each of ten different Mc4r mutations (compared with unaffected family members), including inactivating, constitutively activating and various missense substitutions (Table 1, Fig. 1*a*). Among all 80 non-PWS subjects, there was a negative correlation between ghrelin levels and body-mass index (BMI) ($r = -0.40$, $P < 0.001$) (Fig. 1*b*), indicating that ghrelin is not a major contributor to obesity in

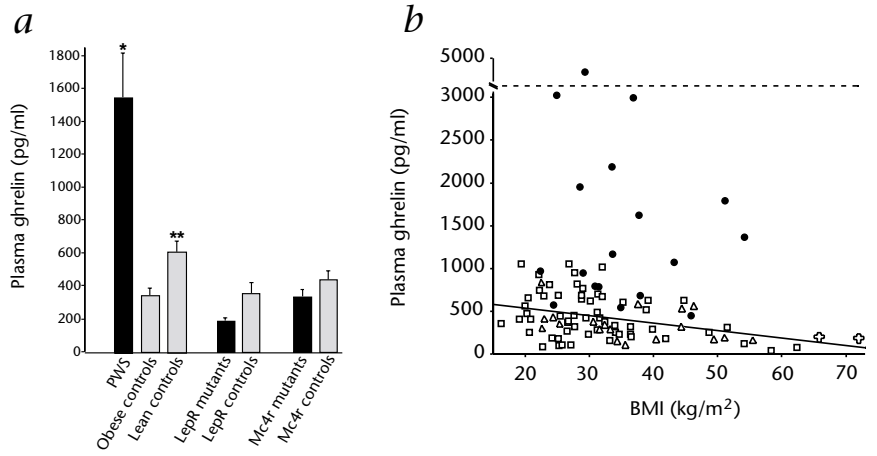

Fig. 1 Obesity from PWS is associated with high circulating ghrelin levels. ***a***, Fasting plasma ghrelin levels were measured with a radio-immunoassay that uses a polyclonal antibody raised against full-length, octanoylated human ghrelin[8]. Study groups are described in Table 1. Dark bars indicate subjects with known mutations; light bars indicate unaffected controls. Values are mean ± s.e.m. *, $P < 0.001$, ANOVA, PWS subjects versus all other groups. **, $P < 0.01$, lean controls versus all other groups except Mc4r controls. ***b***, The normal negative correlation between BMI and plasma ghrelin levels is absent among PWS subjects. Different symbols indicate subjects with PWS (●), a homozygous inactivating LepR mutation (✲), heterozygous Mc4r mutations (△), and all other non-PWS subjects (□). The regression line applies only to individuals without PWS (open symbols, $n = 80$, $r = -0.40$, $P < 0.001$). A negative correlation between BMI and ghrelin levels also exists among all normal subjects without known mutations ($n = 54$, $r = -0.42$, $P = 0.001$). In contrast, there was no correlation between BMI and ghrelin levels in the PWS group ($P = 0.50$). Study protocol approved by the Human Subjects Review Committee of the University of Washington.

these individuals. In contrast, ghrelin levels in PWS subjects were unrelated to BMI and were uniformly above the regression line for non-PWS individuals. Thus, while obesity *per se* is associated with low ghrelin levels, that caused by PWS is associated with elevated ghrelin.

Ghrelin levels in PWS subjects are comparable to or higher than those reported to stimulate appetite and food intake during peripheral ghrelin administration in humans[10] and rodents[17]. Thus, our findings are consistent with a role for hyperghrelinemia in the pathogenesis of hyperphagia in PWS. If elevated ghrelin participates in the GH deficiency of PWS, the effect might be an example of paradoxical override inhibition, which has been described with continuous GH-releasing hormone stimulation of GH[18]. Interventions that lower plasma ghrelin levels, such as gastric bypass surgery[9], warrant consideration in the treatment of obesity from PWS.

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Next steps on ART

To the editor—Your news article "Can WHO provide guidance on HIV drugs for developing countries" (*Nature Med.* **8**, 429; 2002) provided an excellent exegesis of the dilemma facing the World Health Organization (WHO) as it prepared guidelines for the use of antiretroviral therapy (ART) in the developing world.

With the draft of the new guidelines now publicly available (http://www.who.int/HIV_AIDS/first.html), it is clear that the WHO has taken the more difficult and necessary path of creating a set of parameters for scaling-up ART in developing countries. This was a considerable undertaking, and the new guidelines are an important step forward in making ART available more widely. The next step for the WHO is to develop operational models to assist member countries in constructing their own national policies on ART. Providing this technical assistance will require more resources than are currently devoted to HIV/AIDS by WHO.

Of course, helping countries to put these guidelines into practice will require the assistance of more than just the WHO: donor nations and foundations must now support the establishment of AIDS treatment programs as part of a comprehensive response to the global epidemic; drug companies and diagnostic manufacturers must continue to reduce their prices; and the AIDS research community must move to begin operational research on AIDS care in the developing world.

As Justice Edwin Cameron of the Supreme Court of Appeal of South Africa said in a speech at Gay Men's Health Crisis last summer, "This is not a time for indecision and prevarication. It is not a time for preoccupation with supposedly insuperable difficulties. Nor is it a time for indefinite plan-making. It is—especially—not a time for grandiose schemes designed to attain perfection. It is unlikely that in our lifetimes we will attain perfection in Africa. Let us attain something less than perfection in the lives of enough Africans to save them from death by AIDS." (http://www.thebody.com/gmhc/issues/jun01/cameron.html)

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