No Effect of Trp64Arg β_3-Adrenoceptor Polymorphism on the Plasma Leptin Concentration in Pima Indians

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In rodents, administration of leptin promotes β_3-adrenergic stimulation of thermogenesis in brown adipose tissue. Conversely, administration of a β_3-adrenoceptor (β_3-AR) agonist decreases leptin mRNA expression and secretion, suggesting that leptin and sympathetic nervous system activity mediated through the β3-AR comprise a negative-feedback loop. It has recently been proposed that a defect in the β_3-AR in humans may contribute to a resistance to the sympathetically mediated effects of leptin on thermogenesis and lipolysis, thus leading to obesity and type 2 diabetes mellitus. We thus hypothesized that the Trp64Arg variant in the human β3-AR would be associated with elevated plasma leptin concentrations. We studied 101 healthy nondiabetic Pima Indians: 11 Arg64 homozygotes, 35 Trp64 homozygotes, and 55 heterozygotes. The fasting plasma leptin concentration as an absolute value or after adjustment for percent body fat and sex was not associated with the β3-AR genotype. Thus, the data do not support an influence of the Trp64Arg variant on the plasma leptin concentration.

LEPTIN, a 16-kd peptide[1] produced by the adipocyte, is part of a negative-feedback loop involved in the regulation of energy balance. Evidence from rodent studies indicates that leptin administration increases sympathetic nervous outflow universally, stimulating energy expenditure and contributing to weight loss,[2-3] whereas administration of a β_3-adrenoceptor (β_3-AR) agonist decreases leptin mRNA levels[4] and leptin secretion, seemingly independent of a decrease in cell lipid content.[5] In humans, the muscle sympathetic nerve activity (a direct measure of sympathetic nervous outflow) and plasma leptin concentration are positively correlated,[6] suggesting that leptin may also increase sympathetic nervous system activity in humans. However, it is not known whether the β_3-AR variant Trp64Arg, shown to be marginally associated with different facets of obesity and type 2 diabetes mellitus,[7-9] is also associated with the plasma leptin concentration independent of percent body fat. In vitro studies in which the two receptor types, Arg64 and Trp64, were expressed in cell culture have yielded conflicting information about the pharmacological implications of the variant. In one study,[10] the two genotypes, expressed in Chinese hamster ovary (CHO) cells, had similar pharmacological characteristics. Another study,[11] in which the two receptor types were expressed in both CHO-K1 cells and human HEK293 cells, indicated a blunted response to various β-adrenoceptor agonists in the Arg64 type.

The purpose of the present study was to determine whether the Arg64 allele is associated with an elevated plasma leptin concentration in humans. Plasma leptin concentrations were compared in subjects who were homozygous for the Trp64 ("wild-type") allele, homozygous for the Arg64 ("less common type") allele, or heterozygous.

SUBJECTS AND METHODS

Subjects

The plasma leptin concentration and body composition were measured in 101 Pima Indians with previously determined[7] β_3-AR genotype (Table 1). All subjects were healthy, nonmedicated, and nondiabetic according to a 75-g oral glucose tolerance test evaluated according to World Health Organization criteria. The subjects were admitted to the National Institutes of Health Metabolic Research Ward in Phoenix, AZ, and fed a weight-maintenance diet (50% carbohydrate, 30% fat, and 20% protein). Body composition was estimated by underwater weighing with simultaneous determination of residual lung volume. After at least 3 days of the weight-maintenance diet and restrictions on physical activity, samples were collected for determination of the fasting plasma leptin concentration as part of the baseline samples for the oral glucose tolerance test. The study was approved by the Institutional Review Board of the National Institute of Diabetes and Digestive and Kidney Diseases and the Tribal Council of the Gila River Indian Community, and all subjects provided written informed consent.

Analytical Methods

The fasting plasma leptin concentration was measured by a solid-phase sandwich enzyme immunoassay using an affinity-purified polyvalent antibody immobilized in microtiter wells. The assay is sensitive to leptin concentrations of 50 pg/mL. The plasma glucose concentration was determined by the glucose oxidase method (Beckman Instruments, Fullerton, CA) and plasma insulin concentration by radioimmunoassay (Concept 4; ICN, Horsham, PA). Plasma leptin and insulin concentrations were $\log_{10}$-transformed to approximate a normal distribution.

Statistical Analysis

Relationships between variables were determined by Pearson's product-moment correlation or by linear regression analysis to adjust for covariates. The analysis was performed for all subjects and repeated separately for each sex. A P level less than .05 was considered significant.

RESULTS

The body mass, percent body fat, fasting plasma insulin, and fasting plasma glucose did not differ according to β_3-AR genotype (Table 1). The fasting plasma leptin concentration was strongly correlated with percent body fat ($r = .84$, $P = .0001$ in males and $r = .79$, $P = .0001$ in females). By multiple linear regression analysis, β_3-AR genotype was not a significant determinant of the fasting plasma leptin concentration independent of percent body fat and sex. This was also true when the analysis was performed separately for each sex. Furthermore,

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Table 1. Characteristics of the Subjects

Characteristic	Wild-Type Homozygote (Trp/Trp)		Heterozygote (Trp/Arg)		Less Common Type Homozygote (Arg/Arg)	
	Male (n = 19)	Female (n = 16)	Male (n = 39)	Female (n = 16)	Male (n = 7)	Female (n = 4)
Age (yr)	31 ± 5	33 ± 7	31 ± 7	32 ± 6	32 ± 7	27 ± 4
Weight (kg)	104 ± 30	102 ± 18	103 ± 26	85 ± 20	100 ± 17	103 ± 32
Body fat (%)	35 ± 9	50 ± 5	34 ± 8	44 ± 8	34 ± 6	46 ± 6
Body mass index (kg/m^2)	34.8 ± 8.2	40.3 ± 5.6	35.4 ± 8.4	33.4 ± 7.9	33.9 ± 6.1	39.5 ± 11.9
Fasting plasma insulin (mmol/L)	84 (60-108)	198 (156-240)	144 (114-174)	168 (114-222)	132 (66-198)	60 (12-108)
Fasting plasma glucose (mmol/L)	4.8 ± 0.3	5.2 ± 0.4	4.9 ± 0.5	4.9 ± 0.8	4.9 ± 0.3	4.9 ± 0.3
Fasting plasma leptin (ng/mL)	18 (11-25)	52 (42-62)	15 (11-19)	40 (30-50)	14 (10-18)	53 (29-77)

NOTE. Values are the mean ± SD or the mean (95% CI).

the interaction term between β_3-AR genotype and percent body fat was not significant. Figure 1 shows the relation between the percent body fat and fasting plasma leptin concentration by β_3-AR genotype and sex.

DISCUSSION

It has recently been proposed[12] that leptin is part of a feedback loop regulating β_3-AR–mediated sympathetic nervous stimulation of thermogenesis and lipolysis, and that this may explain the association between the Trp64Arg variant in the β_3-AR and obesity. We therefore tested the hypothesis that the Trp64Arg variant in the human β_3-AR is associated with elevated plasma leptin concentrations. In 101 nondiabetic Pima Indians including a substantial number of homozygotes for the rare allele (Arg/Arg), we found no association between the plasma leptin concentration and genotype, whether the leptin concentration was expressed as an absolute value or after adjustment for percent body fat.

The lack of such association could be due to one or more of several reasons. Firstly, expression of β_3-AR mRNA is relatively low in humans compared with rodents.[13] However, since β_3-AR expression in humans is much greater in visceral versus subcutaneous adipose tissue,[14] it is conceivable that an effect of the Trp64Arg variant on visceral adipose tissue leptin production may have effects on the leptin concentration in the portal circulation that cause hepatic insulin resistance or alter pancreatic insulin secretion[15] without detectable effects on the venous leptin concentration. As to rodents, β_3-AR knockout mice have a similar body weight compared with normal mice during high-fat feeding, but a higher fat mass and lower lean mass.[16] In the knockout mice, the positive correlation between the plasma leptin concentration and percent body fat is surprisingly shifted to the right, contrary to the suppressive effect of β_3-adrenergic stimulation on leptin expression. It is possible that an adaptation to the absence of β_3-ARs, such as "cross-talk" with β_1-adrenoceptors,[17] may render the β_3-AR knockout mouse unsuitable for the study of normal physiology. Secondly, leptin secretion is pulsatile.[18] It is possible that the β_3-AR has effects on the pulsatility of leptin secretion that were not detected in the present study, in which only one sample from each subject was used. Thirdly, it is possible that the β_3-AR has effects on the plasma leptin concentration that are not evident in the resting postabsorptive state but may be demonstrated in response to

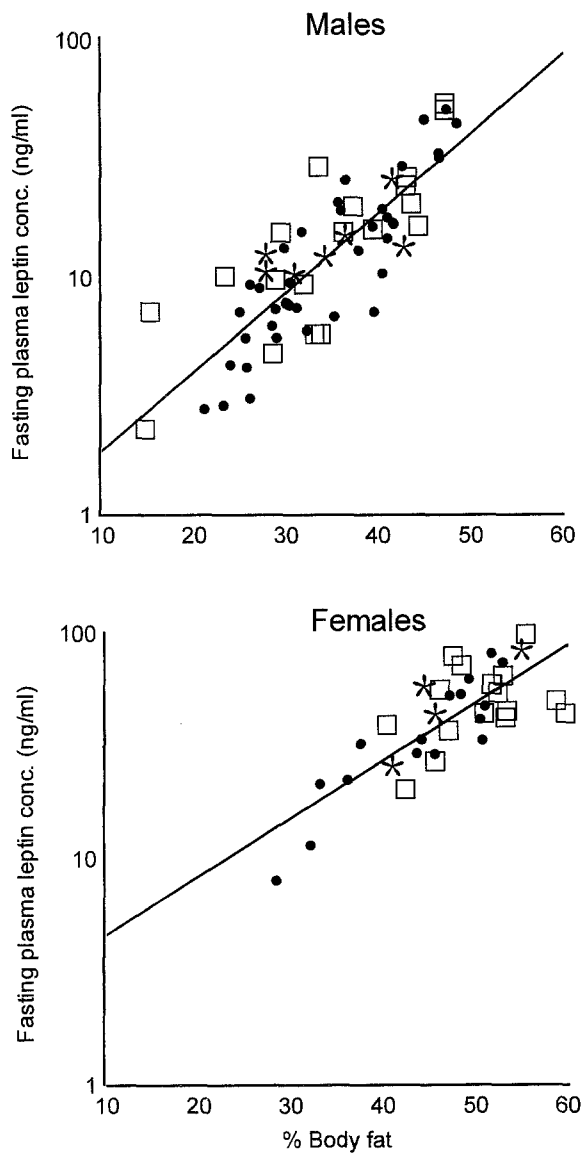

Fig 1. Fasting plasma leptin concentration plotted against % body fat by β3-AR genotype and sex. □, Trp/Trp (wild-type); ●, Trp/Arg (heterozygote); *, Arg/Arg (less common type). Regression lines are for all subjects in that panel.

selective or nonselective β-adrenergic agonists or other stimuli. Finally, the polymorphism in position 64 of the β_3-AR may not have any functional implication, in which case the association between the polymorphism and adverse metabolic factors may be due to linkage disequilibrium with one or more nearby genes influencing metabolism. In vitro studies have produced conflicting information about the pharmacological implications of the variant. In one study,[10] the two genotypes, expressed in CHO cells, had similar pharmacological characteristics. Another study,[11] in which the two receptor types were expressed in both CHO-K1 cells and human HEK293 cells, indicated a blunted response to various β-adrenergic agonists in the Arg64 type.

In conclusion, the present data obtained in a large group of Pima Indians including a substantial number of individuals homozygous for the rare allele (Arg/Arg) do not support an influence of the Trp64Arg variant on the plasma leptin concentration in humans.

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