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Low Plasma Leptin in Response to Dietary Fat in Diabetes- and Obesity-Prone Mice

Richard S. Surwit, Ann E. Petro, Priti Parekh, and Sheila Collins

Despite the fact that mutations resulting in the absence of leptin or its receptor have been associated with severe obesity and diabetes, such mutations do not appear to be responsible for most human obesity. Indeed, diet-induced obesity in animals and humans has been characterized by hyperleptinemia. This has been interpreted as evidence for leptin resistance. However, no careful longitudinal studies evaluating the role of leptin in the development of obesity exist. We report a series of studies in A/J and C57BL/6J (B/6) mice that demonstrate a direct relationship between the ability to increase plasma leptin levels in response to a high-fat diet and resistance to the subsequent development of obesity and diabetes. While leptin levels are similar in lean, low-fat-fed A/J and B/6 mice, the effects of a high-fat diet on plasma leptin differ dramatically between the two strains. After 4 weeks of high-fat feeding, leptin levels in A/J mice increased 10-fold, and this elevated level was maintained independent of weight gain throughout a 14-week feeding period. However, in B/6 mice, leptin levels remained at least twofold lower and only rose very gradually along with a significant increase in adiposity, hyperglycemia, and hyperinsulinemia. These differences in the response of leptin to diet are independent of food intake and plasma insulin levels during the 1st month of feeding. Further, we demonstrated that leptin administration did not influence the expression of the novel uncoupling protein UCP2, which also responds to dietary fat. From these results, we suggest that the response of leptin to fat feeding may be an important predictor of the development of subsequent obesity.
***Diabetes* 46:1516–1520, 1997**

Obesity in mutant rodents has been associated with decreased leptin production or absence of functional leptin receptors (1–4), but these abnormalities have not been found in humans (5–9). In fact, nonmutant obese animals and humans typically show elevated plasma leptin levels and increased expression of leptin mRNA in adipose tissue (5–10). Several animal models of diet-induced obesity have been developed by manipulating nutritional variables in different inbred strains (11–14). These models provide the opportunity to evaluate the interaction of diet and genetic background on the longitudinal development of obesity and associated metabolic parameters. One of the most well characterized is the C57BL/6J (B/6) mouse, the background strain on which the obese mutation commonly resides (15). The B/6 mouse remains lean and otherwise normal on low-fat diets such as standard rodent feed. However, after being raised on a high-fat diet, it develops severe obesity, hyperglycemia, hyperinsulinemia, altered β-cell function, and hypertension in adulthood (11,13,14,16–18). In contrast, strains such as A/J gain weight on a high-fat diet in direct proportion to increased caloric intake, while B/6 mice show both increased caloric intake and increased feed efficiency (body weight gain per kilocalories consumed) in response to fat (14). Furthermore, obesity in B/6 mice results both from adipocyte hyperplasia and from hypertrophy of existing adipocytes, while obesity in A/J mice is associated only with adipocyte hypertrophy (13,14). We now report the first association of decreased plasma leptin levels in a nonmutant animal model of diet-induced obesity. We have identified distinct patterns of diet-dependent leptin levels in two inbred strains of mice that show differential weight gain on high-fat diets.

RESEARCH DESIGN AND METHODS

Animals. Male C57BL/6J (B/6) and A/J mice were obtained from the Jackson Laboratory (Bar Harbor, ME) at 4 weeks of age. The animals were housed five per cage in a temperature-controlled room with a 12-h light/dark cycle. The mice were allowed ad libitum access to water and one of two diets: a high-fat diet (58% of calories from fat) or a low-fat diet (10.5% of calories from fat). Diets were manufactured by Research Diets (New Brunswick, NJ). All diets contained 16% protein and met the American Institute of Nutrition requirements for mice with regard to mineral and vitamin content. The specific composition of the diets has been described in detail previously (14). Body weight was determined biweekly. Animals were housed individually for 24 h once per week for food intake measurements.

Assays. After 8 h of food deprivation, blood samples were collected via retro-orbital sinus puncture in unanesthetized animals. Plasma glucose concentrations were determined using a Beckman Glucose Analyzer 2. Plasma insulin and leptin were measured with double-antibody radioimmunoassay kits (Linco Research, St. Louis, MO) based on a rat standard (for insulin) and a mouse standard (for leptin).

Body temperature. Animals were allowed a 1-week acclimation period before the onset of the study. The room temperature was maintained at 22.3 ± 0.7°C. Body temperature was assessed at the beginning of the light cycle. Rectal temperatures were measured with a thermocouple microprobe (Physitemp, Clifton, NJ). The probe was inserted 10 mm into the rectum, and the temperature was recorded after 15 s.

Data analysis. Comparisons between strains for single factors were performed by unpaired *t* test. Data comparing diets and strains were analyzed by one-way

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Received for publication 6 January 1997 and accepted in revised form 9 July 1997.

UCP, uncoupling protein.

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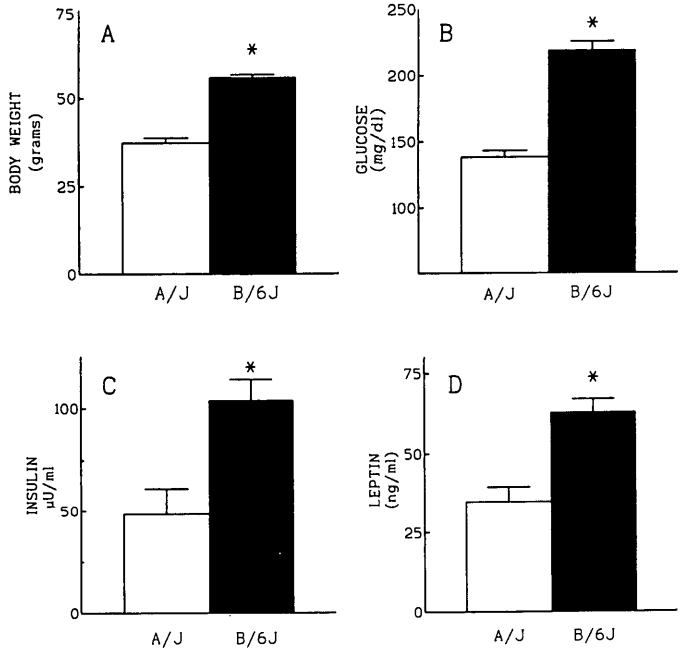

FIG. 1. Differences between A/J and B/6 mice after 8 months on a high-fat diet. Body weight (*A*), plasma glucose (*B*), plasma insulin (*C*), and plasma leptin (*D*) values of A/J and B/6 mice (n = 10/group) after 8 months on a high-fat diet. The values shown are means ± SE. *P < 0.001.

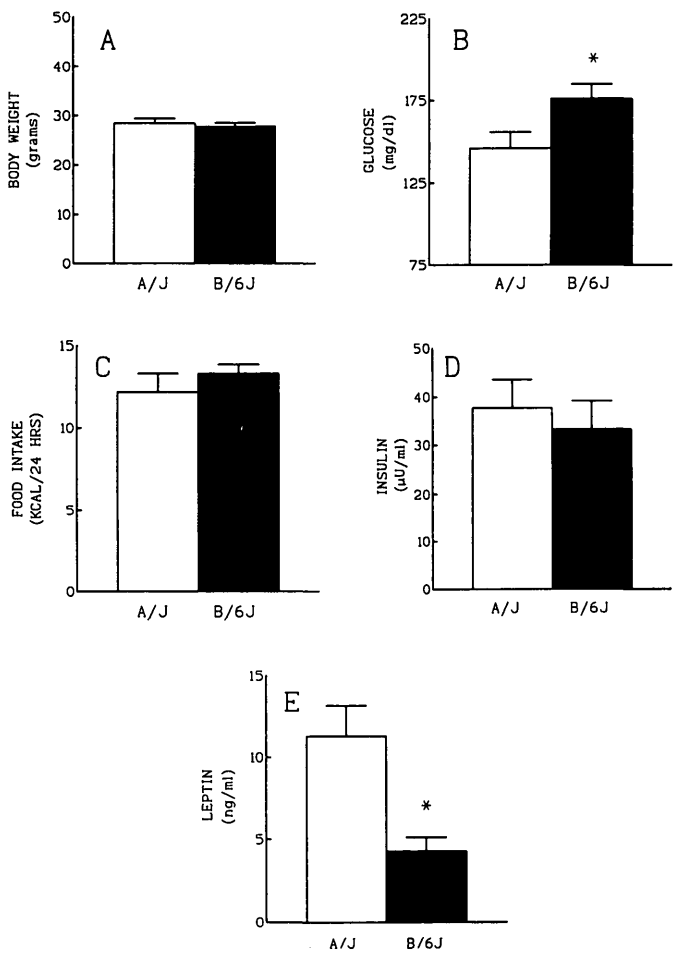

FIG. 2. Differences between A/J and B/6 mice after 1 month on a high-fat diet. Body weight (n = 9 A/J; n = 10 B/6) (*A*), plasma glucose (n = 9 A/J; n = 10 B/6) (*B*), plasma insulin (n = 9 A/J; n = 10 B/6) (*C*), food intake (n = 9, A/J; n = 10, B/6) (*D*), and plasma leptin (n = 7, A/7; n = 9, B/6) (*E*) of A/J and B/6 mice after 1 month on a high-fat diet. Values shown are means ± SE. *P < 0.001.

analysis of variance followed by post hoc tests (Newman-Keuls multiple comparisons and Bartlett's test for equal variances). For time-course studies, a repeated measures analysis was used, followed by individual t test analyses.

RESULTS

Experiment 1. Ten A/J and 10 B/6 mice were weaned onto the high-fat diet at 1 month of age, as previously described (14), and maintained on this diet for 8 months. As is characteristic of B/6 mice, they became significantly obese, attaining body weights of 58 ± 0.9 g, while A/J mice weighed 37 ± 1.5 g (Fig. 1*A*). B/6 mice are also hyperglycemic (Fig. 1*B*) and hyperinsulinemic (Fig. 1*C*) and have higher levels of plasma leptin than do A/J mice (Fig. 1*D*). In this regard, B/6 mice resemble humans with diabetes and obesity. Because we have often observed that the earliest metabolic abnormalities can be detected after 1 month on this diet (unpublished observations), we began a series of experiments to study the early effects of high-fat feeding on plasma leptin levels.

Experiment 2. In this study, animals of both strains were examined after consuming the high-fat diet for 1 month. Although B/6 mice are mildly hyperglycemic relative to A/J mice, body weight, food intake, and plasma insulin level in both strains are similar after 1 month on this diet (Fig. 2*A–D*). Unexpectedly, we found that plasma leptin levels in A/J mice were elevated 10-fold over those of their low-fat-fed counterparts and were at least 2-fold higher than those of high-fat-fed B/6 mice (Fig. 2*E*). Interestingly, despite a depressed level of plasma leptin in B/6 mice, food intake was not different in the two strains (Fig. 2*C*). Thus, the effects of leptin on subsequent obesity cannot be explained simply by differences in food intake.

Experiment 3. In this study, 10 animals of both strains were weaned onto the low-fat diet at 1 month of age. After 2 weeks on the low-fat diet, all animals were switched to the high-fat diet described above and followed for 14 weeks. A/J and B/J mice had comparable body weights and glucose, insulin, and leptin levels on a low-fat diet (week 0 in Fig. 3*A–D*). However, after 2 weeks on the high-fat diet, B/6 mice showed higher weight, glucose, and insulin (Fig. 3*A–C*). Again, we found that plasma leptin levels in A/J mice were elevated at least twofold over those seen in B/6 mice. Leptin levels in B/6 mice only exceeded those of A/J mice after the development of significant adiposity. Although insulin has been reported to increase leptin expression in cultured adipocytes in vitro (19–21) or upon exogenous insulin administration to mice (22), our observations suggest that this does not necessarily occur in vivo.

Experiment 4. To confirm the observation that strain differences in plasma leptin were dependent on diet, a fourth study was conducted in B/6 and A/J mice that were raised on the low-fat diet and followed for 14 weeks. The body weights and plasma leptin levels are shown in Fig. 4. Although A/J mice showed slightly higher leptin levels than did B/6 mice, the differences were much smaller than those observed with the high-fat diet.

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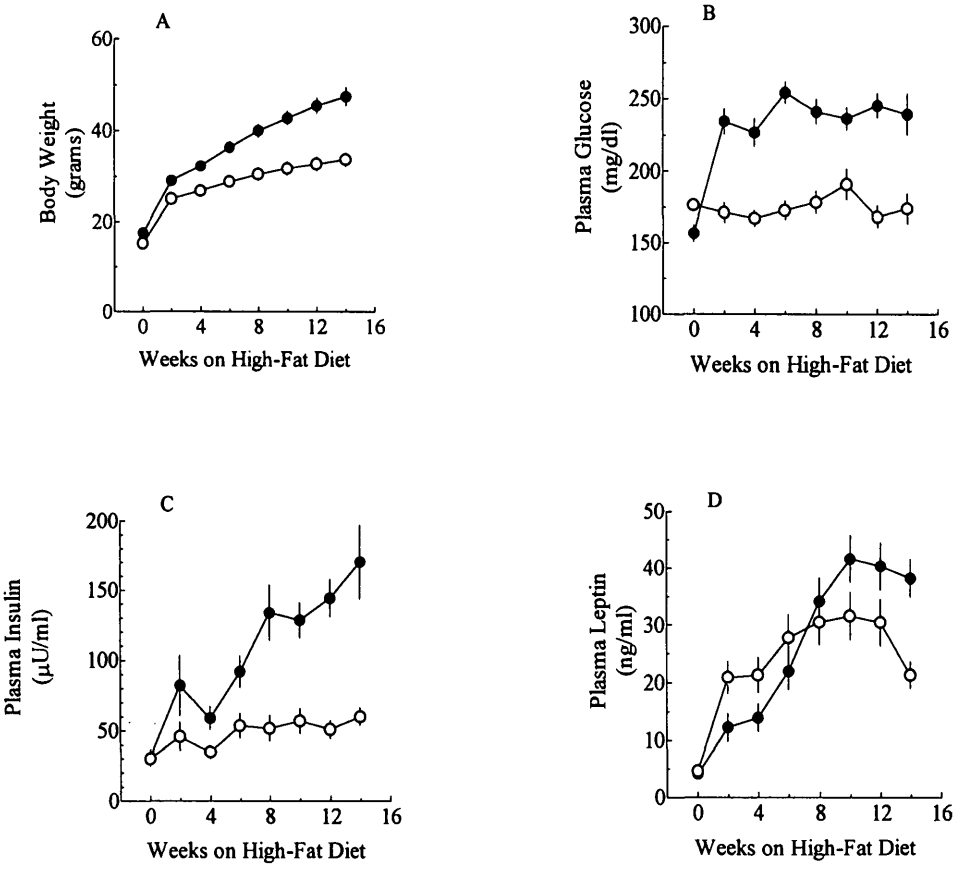

FIG. 3. Biweekly analysis of the effects of a high-fat diet on body weight (*A*), plasma glucose (*B*), plasma insulin (*C*), and plasma leptin (*D*) in A/J (○) and B/6 (●) mice. B/6 mice showed significantly higher body weight at 2 and 14 weeks ($P < 0.003$), significantly higher glucose at 2 and 14 weeks ($P < 0.01$), and significantly higher insulin at 14 weeks ($P < 0.02$). A/J mice, however, showed higher leptin at 2 weeks ($P < 0.05$), while B/6 mice showed higher leptin at 14 weeks ($P < 0.003$).

Experiment 5. We previously observed that leptin administration to C57BL/6J *ob/ob* mice increases catecholamine turnover in brown adipose tissue (23). Therefore, because a diet-induced rise in leptin may contribute to increased thermogenesis, we measured core temperatures of 10 mice from each strain after 2 weeks on the low-fat or high-fat diet. While there were no significant differences in core temperature between A/J and B/6 mice on the low-fat diet (A/J: 35.7 ± 0.15°C; B/6: 36.0 ± 0.10°C; $P > 0.05$), high-fat feeding resulted in a significant increase in core temperature in A/J mice, but not in B/6 mice (Fig. 5).

Experiment 6. Because A/J mice show increased expression of the novel uncoupling protein UCP2 (24) in white adipocytes after the introduction of high-fat feeding, while B/6 mice do not, we performed a final study to determine if leptin has a role in regulating UCP2 expression. Twelve A/J and 12 B/6 mice were fed the low-fat diet and given twice-daily injections of 20 μg leptin or saline vehicle for 4 days. On the 5th day, animals were killed by cervical dislocation, and white adipose samples were collected from the epididymal fat pad. UCP2 mRNA was measured by Northern blot as previously described (24). As can be seen in Fig. 6, A/J mice showed higher levels of UCP2 expression than did B/6 mice, but there were no differences in UCP2 expression in animals receiving leptin. While leptin supplementation did not affect weight gain in A/J mice, B/6 mice receiving leptin

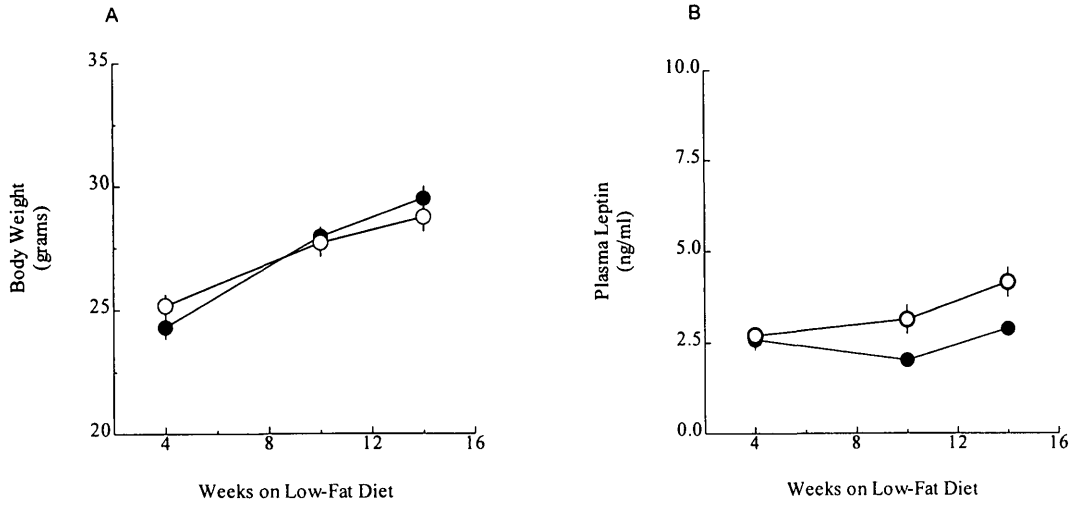

FIG. 4. Body weight (*A*) and plasma leptin levels (*B*) in low-fat-fed A/J (○) and B/6 (●) mice. Values shown are means ± SE. The number of animals varied from 8 to 10 per group at each time point. There were no significant differences between the strains for body weight ($P = 0.98$) or leptin ($P = 0.17$).

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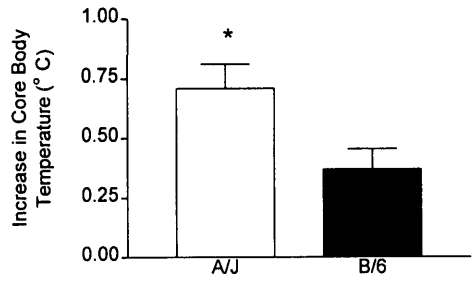

FIG. 5. Relative increase in body temperature in A/J and B/6 mice caused by high-fat feeding. Ten animals from each strain were allowed a 1-week acclimation period before the onset of the study. Core temperatures were measured after 2 weeks on a low-fat or high-fat diet. The data shown represent the increase in body temperature in animals fed the high-fat diet. Data shown are means ± SE.

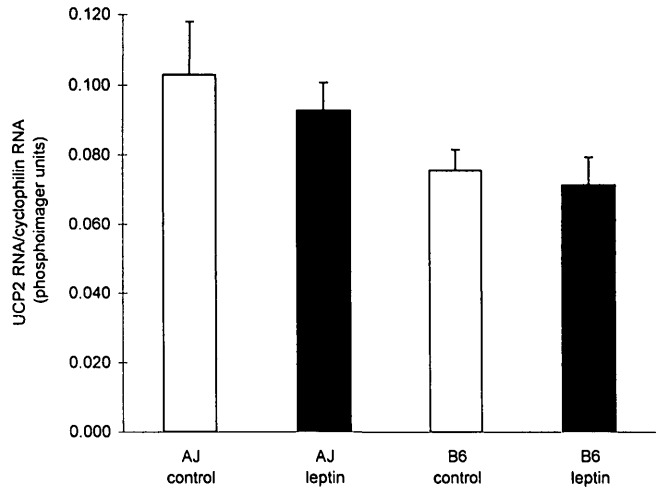

FIG. 6. Effect of exogenous leptin on UCP2 mRNA levels in white adipose tissue. Twelve animals from each strain were fed a low-fat diet. The animals were injected twice daily for 4 days with either leptin (20 mg) or saline control. Epididymal white adipose tissue RNA was electrophoresed and a Northern blot prepared. The blot was probed with ^{32}P-labeled clone 129216 (human UCP2) and with cyclophilin. Amounts of UCP2 mRNA were determined with a Molecular Dynamics phosphorimager and were normalized with cyclophilin mRNA levels. Data shown are mean ± SE.

gained less weight than did B/6 mice receiving vehicle ($P < 0.05$). Therefore, the dose of leptin used was physiologically effective.

DISCUSSION

While the discovery of leptin has elucidated a new feedback loop in the hormonal control of adipocyte function, this new hormone has only been causally associated with obesity in murine mutations that lack an intact gene for leptin or for its receptor (1,2). The role of leptin in more common forms of obesity remains a mystery (5). In this study, we demonstrated reduced plasma leptin in response to fat feeding in a diet-induced model of diabetes and obesity that is remarkably similar to human forms of these disorders (11,13,14,16,17). Previous studies reporting elevated leptin in obese individuals would seem to contradict the ability of leptin to increase energy expenditure and reduce food intake when administered to mice (25–27). However, the differential leptin response to a high-fat diet that we observed in these obesity-prone and obesity-resistant strains of mice may point to an explanation of this inconsistency. Our results show that obesity-resistant A/J mice respond to high-fat diets immediately with a robust rise in plasma leptin. Because leptin can increase sympathetic outflow to brown fat (23), this response could be related to the phenomenon of diet-induced thermogenesis (28,29), in which activation of uncoupling protein (UCP1) in brown adipose tissue increases metabolic rate and promotes dissipation of excess caloric intake. Thus, elevated plasma leptin in response to high-fat feeding may be responsible for the lower feed efficiency and plasma glucose level in the A/J mouse on a high-fat diet. Indeed, this hypothesis is supported by the observation that high-fat feeding is associated with increased core temperature in A/J, but not B/6 mice. Alternatively, increased body temperature in response to fat feeding may also be due, in part, to the increased expression of the novel uncoupling protein UCP2 in fat-fed A/J as opposed to B/6 mice (24). However, administration of leptin did not significantly increase UCP2 expression in either A/J or B/6 mice. Thus, it is unlikely that previously observed differences in UCP2 expression after fat feeding (24) are related to the effects of dietary fat on leptin secretion. It is possible that UCP2 expression after the introduction of a high-fat diet may be part of the signaling system that results in the different leptin levels in A/J and B/6 mice reported here.

Although we observed differences in the absolute levels of plasma leptin in A/J and B/6 mice among the individual experiments performed, the qualitative differences between the A/J and B/6 mice in response to fat feeding were consistent. At this time, we do not know the reason for these differences, but they may be due to the exact ages at which high-fat feeding was initiated. For example, in experiment 3, animals were introduced to the high-fat diet at 2 months of age (first weaned onto the low-fat diet for 1 month), while in experiment 2, animals were directly weaned onto the high-fat diet at 1 month of age.

Insulin has been postulated to be a necessary stimulus for leptin release, but our data suggest that this is not a simple phenomenon. While hyperleptinemia develops with hyperinsulinemia in B/6 mice, A/J mice show higher initial leptin levels in the absence of elevated insulin. It is also not likely that the failure of B/6 mice to secrete leptin is a function of insulin resistance because leptin increases in B/6 mice over time despite the fact that the glucose-to-insulin ratio reflects increasing insulin resistance in this strain. We also note that during the first few weeks of a high-fat diet, food intake is the same between the two strains even though plasma leptin levels are much lower in the B/6 mice.

Our findings are consistent with those in a recent report of Ravussin et al. (30), in which lower plasma leptin levels were associated with greater weight gain in a sample of Pima Indians. It has been noted previously that Pima Indians have lower sympathetic nervous system activity than do Caucasians and that those with lower activity have a greater amount of body fat than do those with higher activity (31). This suggests that the relationship of plasma leptin to subsequent weight gain is biologically conserved and of potential clinical significance.

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ACKNOWLEDGMENTS

This work was supported in part by National Institutes of Health Grants R29DK46793 (S.C.) and K05MH00303 (R.S.S.).

We thank Paul Blackwelder and Kiefer W. Daniel for assistance with animal care and isolation of plasma and tissue samples from mice.

REFERENCES

1. Zhang Y, Proenca R, Maffei M, Barone M, Leopold L, Friedman JM: Positional cloning of the mouse obese gene and its human homologue. *Nature* 372:425–479, 1994
2. Tartaglia LA, Dembski M, Weng X, Deng N, Culpepper J, Devos R, Richards GJ, Campfield LA, Clark FT, Deeds J, Muir C, Sanker S, Moriarty A, Moore KJ, Smutko JS, Mays GG, Woolf EA, Monroe CA, Tepper RI: Identification and expression cloning of a leptin receptor, OB-R. *Cell* 83:1263–1271, 1995
3. Lee G-H; Proenca R, Montez JM, Carrol, KM, Darbishzadeh JG, Lee JI, Friedman JM: Abnormal splicing of the leptin receptor in diabetic mice. *Nature* 379:632–635, 1996
4. Chen H, Charlat O, Tartaglia LA, Woolf, EA, Weng Z, Ellis SJ, Lakery ND, Culpepper J, Moore KJ, Breitbart TE, Duyk GM, Tepper RI, Morgenstern JP: Evidence that the diabetes gene encodes the leptin receptor: identification of a mutation in the leptin receptor gene in db/db mice. *Cell* 84:491–495, 1996
5. Considine RV, Considine EL, Williams CJ, Nyce MR, Magosin SA, Bauer TL, Rosato EL, Colberg J, Caro JF: Evidence against either a premature stop codon or the absence of obese gene mRNA in human obesity. *J Clin Invest* 95:2986–2988, 1995
6. Considine RV, Considine EL, Williams CJ, Hyde TM, Caro JF: The hypothalamic leptin receptor in humans: identification of incidental sequence polymorphisms and absence of the *db/db* mouse and *fa/fa* rat mutations. *Diabetes* 19:992–994, 1996
7. Maffei M, Fei H, Lee G-H, Dani C, Leroy P, Zhang Y, Proenca R, Negrel R, Ailhaud G, Friedman JM: Increased expression in adipocytes of ob RNA in mice with lesions of the hypothalamus and with mutations at the *db* locus. *Nature Genet* 92:6957–6960, 1995
8. Ogawa Y, Masuzki H, Isse N, Okazaki T, Mori K, Shigemoto M, Satoh N, Tamura N, Hosoda K, Yoshimasa Y, Jingami H, Kawada T, Nakao K: Molecular cloning of rat *obese* cDNA and augmented gene expression in genetically obese Zucker Fatty (*fa/fa*) rats. *J Clin Invest* 96:1647–1652, 1995
9. Considine RV, Sinha MK, Heiman ML, Kriauciunas A, Stephens TW, Nyce MR, Ohannesian JP, Marco CC, McKee LJ, Bauer TL, Caro JF: Serum immunoreactive-leptin concentrations in normal-weight and obese humans. *N Engl J Med* 334:292–295, 1996
10. Collins S, Surwit RS: Pharmacological manipulation of ob gene expression in a dietary model of obesity. *J Biol Chem* 271:9437–9440, 1996
11. Surwit RS, Kuhn CM, Cochrane C, McCubbin JA, Feinglos MN: Diet-induced type II diabetes in C57BL/6J mice. *Diabetes* 37:1163–1167, 1988
12. West DB, Boozer CN, Moody DL, Atkinson RL: Dietary obesity in nine inbred mouse strains. *Am J Physiol* 262:R1025–R1032, 1992
13. Rebuffe-Scrive M, Surwit R, Feinglos M, Kuhn C, Rodin J: Regional fat distribution and metabolism in a new mouse model (C57BL/6J) of non-insulin-dependent diabetes mellitus. *Metab Clin Exp* 42:1405–1409, 1993
14. Surwit RS, Feinglos MN, Rodin J, Sutherland A, Petro AE, Opara EC, Kuhn CM, Rebuffe-Scrive M: Differential effects of fat and sucrose on the development of obesity and diabetes in C57BL/6J and A/J mice. *Metabolism* 44:645–651, 1995
15. Coleman DL: Diabetes-obesity syndromes in mice. *Diabetes* 31:1–6, 1982
16. Mills E, Kuhn CM, Feinglos MN, Surwit RS: Hypertension in C57BL/6J mouse model of non-insulin-dependent diabetes mellitus. *Am J Physiol* 264:R73–R78, 1993
17. Lee SK, Opara EC, Surwit RS, Feinglos MN, Akwari OE: Defective glucose-stimulated insulin release from perifused islets of C57BL/6J mice. *Pancreas* 11:206–211, 1995
18. Wencel HE, Smothers C, Opara EC, Kuhn CM, Feinglos MS, Surwit RS: Impaired second phase insulin response of diabetes-prone C57BL/6J mouse islets. *Physiol Behav* 57:1215–1220, 1995
19. Rentsch J, Chiesi M: Regulation of ob gene mRNA levels in cultured adipocytes. *FEBS Lett* 379:55–59, 1996
20. Leroy P, Dessolin S, Villageois P, Moon BC, Friedman JM, Ailhaud G, Dani C: Expression of ob gene in adipose cells. *J Biol Chem* 271:2365–2368, 1996
21. Gettys TW, Harkness PJ, Watson PM: The beta3-adrenergic receptor inhibits insulin-stimulated leptin secretion from isolated rat adipocytes. *Endocrinology* 137:4054–4057, 1996
22. Saladin R, de Vos P, Guerre-Millo M, Leturque A, Girard J, Staels B, Auwerx J: Transient increase in obese gene expression after food intake or insulin administration. *Nature* 377:527–529, 1995
23. Collins S, Kuhn CM, Petro AE, Swick AG, Chrunyk BA, Surwit RS: Role of leptin in fat regulation. *Nature* 380:677, 1996
24. Fleury C, Neverova M, Collins S, Raimbault S, Champigny O, Levi-Meyrueis C, Bouillard F, Seldin MF, Surwit RS, Ricquier D, Warden CH: Uncoupling protein-2: a novel gene linked to obesity and diabetes. *Nature Genet* 15:269–272, 1997
25. Pelleymounter MA, Cullen MJ, Baker MB, Hecht R, Winters D, Boone T, Collins F: Effects of the obese gene product on body weight regulation in ob/ob mice. *Science* 269:540–543, 1995
26. Halaas JL, Gajiwala KS, Maffei M, Cohen SL, Chait BT, Rabinowitz D, Lallone RL, Burley SK, Friedman JM: Weight-reducing effects of the plasma protein encoded by the obese gene. *Science* 269:543–546, 1995
27. Campfield LA, Smith FJ, Guisez Y, Devos R, Burn P: Recombinant mouse OB protein: evidence for a peripheral signal linking adiposity and central neural networks. *Science* 269:546–549, 1995
28. Rothwell NJ, Stock MJ: A role for brown adipose tissue in diet-induced thermogenesis. *Nature* 281:31–35, 1979
29. Brooks SL, Rothwell NJ, Stock MJ, Goodbody AE, Trayhurn P: Increased proton conductance pathway in brown adipose tissue mitochondria of rats exhibiting diet-induced thermogenesis. *Nature* 286:274–276, 1980
30. Ravussin E, Pratley RE, Maffei M, Wang H, Friedman JM, Bennett PH, Bogardus C: Low plasma leptin concentrations precede weight gain in Pima Indians. *Nature Med* 3:238–240, 1997
31. Spraul M, Ravussin E, Fontwieille AM, Rising R, Larson DE, Anderson EA: Reduced sympathetic nervous system activity: a potential mechanism predisposing to body weight gain. *J Clin Invest* 92:1730–1735, 1993

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