Kinin B_1 Receptor Deficiency Leads to Leptin Hypersensitivity and Resistance to Obesity

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OBJECTIVE—Kinins mediate pathophysiological processes related to hypertension, pain, and inflammation through the activation of two G-protein–coupled receptors, named B_1 and B_2. Although these peptides have been related to glucose homeostasis, their effects on energy balance are still unknown.

RESEARCH DESIGN AND METHODS—Using genetic and pharmacological strategies to abrogate the kinin B_1 receptor in different animal models of obesity, here we present evidence of a novel role for kinins in the regulation of satiety and adiposity.

RESULTS—Kinin B_1 receptor deficiency in mice ($B_1^{-/-}$) resulted in less fat content, hypoleptinemia, increased leptin sensitivity, and robust protection against high-fat diet–induced weight gain. Under high-fat diet, $B_1^{-/-}$ also exhibited reduced food intake, improved lipid oxidation, and increased energy expenditure. Surprisingly, B_1 receptor deficiency was not able to decrease food intake and adiposity in obese mice lacking leptin (*ob/ob*-$B_1^{-/-}$). However, *ob/ob*-$B_1^{-/-}$ mice were more responsive to the effects of exogenous leptin on body weight and food intake, suggesting that B_1 receptors may be dependent on leptin to display their metabolic roles. Finally, inhibition of weight gain and food intake by B_1 receptor ablation was pharmacologically confirmed by long-term administration of the kinin B_1 receptor antagonist SSR240612 to mice under high-fat diet.

CONCLUSIONS—Our data suggest that kinin B_1 receptors participate in the regulation of the energy balance via a mechanism that could involve the modulation of leptin sensitivity. ***Diabetes*** **57:1491–1500, 2008**

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$B_1^{-/-}$, kinin B_1 receptor knockout mice; LPS, lipopolysaccharide; NF-κB, nuclear factor-κB; *ob/ob*, obese mice lacking leptin; SOCS3, suppressor of cytokine signaling-3; TNF-α, tumor necrosis factor-α.

According to the World Health Organization, one billion people worldwide are overweight, and at least 300 million are clinically obese (www.who.int). Besides the negative social stigma carried by obese individuals, obesity can cause or exacerbate many health problems, such as type 2 diabetes, cardiovascular diseases, and some cancers (1,2). Thus, obesity is associated with increased mortality (3) and accounts for up to 7.8% of the total health care expenses in developed countries (4).

In 1994, Zhang et al. (5) contributed significantly to our understanding of the endocrine mechanisms underlying the regulation of body weight. The group identified a null mutation in the *ob* gene that led mice to a phenotype of severe obesity. The functions of the *ob* gene product, a 16-kDa cytokine called leptin, were further characterized by several subsequent studies. Traditionally, it has been shown that leptin acts in the arcuate nucleus of the hypothalamus, inhibiting food intake and promoting energy expenditure (6). Lately, it has been shown that leptin has a broad spectrum of action in many areas of the central nervous system and peripheral organs. For instance, it was shown that leptin prevents lipotoxicity in liver, skeletal muscle, and pancreas by direct inhibition of triglyceride formation and stimulation of free fatty acid oxidation (7). Mice lacking leptin (*ob/ob*) show hyperphagia, insulin resistance, hypothermia, reduced sympathetic activation, and infertility, parameters that can be restored after administration of exogenous leptin (6,8). Despite the powerful metabolic actions of leptin, the therapeutic use of this hormone was initially frustrated by observations that plasma leptin levels were in fact elevated in obese individuals (9), which indicated that obesity in humans was accompanied by leptin resistance rather than hypoleptinemia. More recently, various mechanisms for leptin resistance in obesity have been proposed. The two main hypotheses are the saturation of the leptin transport through the blood-brain barrier (10,11) and the failure of components of the leptin receptor (Ob-Rb) signaling pathway (12). It was shown, for example, that the overexpression of the suppressor of cytokine signaling-3 (SOCS3) caused leptin resistance in different animal models of obesity because it resulted in a strong reduction of the Ob-Rb signal transduction (13). Therefore, the identification of alternative factors that potentiate leptin signaling may lead to useful therapeutic approaches to treat obesity.

The kallikrein-kinin system represents a group of proteins involved in the control of blood pressure, gastroin-

testinal tract mobility, inflammation, and pain induction (14,15). In this system, precursor proteins called kininogens are cleaved by kallikreins to release kinins, which can further be cleaved by carboxypeptidases to give rise to des-Arg-kinins. The main active kinins are bradykinin and its metabolite des-Arg^9-bradykinin. Bradykinin and des-Arg^9-bradykinin use distinct G-protein–coupled receptors to exert their effects. Whereas bradykinin interacts with the B_2 receptor subtype, des-Arg^9-bradykinin has higher affinity for the B_1 receptor (14). Kinin B_2 receptors are constitutively expressed in most tissues and exert most functions attributed to the kallikrein-kinin system, including vasodilation and salt excretion (14,16). Conversely, the kinin B_1 receptor is thought to be strongly induced by inflammatory stimuli and to mediate processes of injury and pain (14,15,17). Accordingly, kinin B_1 receptor knockout mice ($B_1^{-/-}$) are hypoalgesic, are less responsive to lipopolysaccharide (LPS)-induced hypotension (18), and present inhibition of neutrophil migration into inflamed tissue (19).

Evidence that kinins induce insulin sensitivity in adipocytes (20) and control insulin released by pancreatic cells (21,22) indicates that the kallikrein-kinin system also participates in metabolic processes. However, the physiological relevance of this system to energy homeostasis and the contributions of the kinin B_1 receptor to these processes are still not well understood. Recently, our group showed that the B_1 receptor mRNA is upregulated in the white adipose tissue, liver, and hypothalamus of *ob/ob* mice (23), suggesting for the first time a correlation between obesity and the kinin B_1 receptor. Furthermore, we observed that des-Arg^9-bradykinin administration in mice is able to increase blood leptin levels (24). In the present report, we examined the consequences of B_1 receptor ablation on the etiopathology of obesity using two animal models, the high-fat diet–induced obesity and the obese *ob/ob* mice. In addition, we evaluated the effect of pharmacological inhibition of B_1 receptors on weight gain and food intake of mice under high-fat diet. The results of these studies strongly support a role for the kinin B_1 receptor in controlling body weight and fat accumulation through a mechanism that may involve the modulation of leptin signaling.

RESEARCH DESIGN AND METHODS

$B_1^{-/-}$ mice (18) used in the experiments were originated from 10 generations' backcrossing of an initially mixed genetic background (129/Sv and C57Bl/6) with C57Bl/6 mice (Taconic, Germantown, NY). Therefore, C57Bl/6 animals were used as their controls. The *ob/ob*-$B_1^{-/-}$ strain was generated by crossbreeding C57Bl/6 $ob^{+/-}$ mice (The Jackson Laboratories, Bar Harbor, ME) with $B_1^{-/-}$ mice. Animals have been obtained from the Universidade Federal de São Paulo (Brazil), from the Max-Delbrück-Center for Molecular Medicine (Berlin-Buch, Germany), and from the Animal House (Toulouse, France). All experiments reported have been conducted as stated in the National Institutes of Health Guide for the Care and Use of Laboratory Animals (Institute of Laboratory Animal Resources, National Academy Press, Washington, DC, 1996) and approved by a local committee. Animals were maintained on standard mouse chow at 22°C on a 12-h light-dark cycle with ad libitum access to food and tap water. Food consumption and body weight were monitored weekly in individualized animals. In all experiments, 12- to 18-week-old males were used.

High-fat diet treatment. Mice were fed either standard diet (10% kcal fat) or high-fat diet (45% kcal fat) (Research Diets, New Brunswick, NJ) for 9 weeks. After the treatment, mice were killed for blood and tissue collection. The sera were separated for leptin and insulin quantification with ELISA kits (R&D Systems [Minneapolis, MN] and Millipore [Billerica, MA], respectively). Tissues explants were weighed and used for adipocyte isolation, or frozen for protein, RNA, or triglycerides extraction.

Body composition analysis. Total body fat was estimated by dual-energy X-ray absorptiometry using a Hologic QDR 4500 scanner (Hologic, Waltham, MA) as described previously (25).

Adipose tissue cellularity. Isolation of fat cells and calculation of adipocyte volume and number in epididymal fat depots were performed as previously described (26). Briefly, images of isolated adipocytes were acquired from a light microscope fitted with a camera, and the average cellular volume was estimated by measuring the diameter of at least 100 adipocytes per animal using the software ImageJ (http://rsb.info.nih.gov/ij/). Fat cell number was calculated by dividing the adipose tissue triglyceride content by the average adipocyte lipid content, obtained by the multiplication of the average fat cell volume by the triolein density (0.92).

Gene and protein expression. Please refer to the online appendix (available at http://dx.doi.org/10.2337/db07-1508).

Energy expenditure. In vivo indirect open circuit calorimetry was performed as previously described (26). Briefly, respiratory exchanges and spontaneous activity signals of freely moving mice were computed at 10-s intervals using a computer-assisted data acquisition program (ADDENFI metabolic chamber prototype). Mice were housed individually in the metabolic cages at 10:00 A.M. without food, and the postabsorptive resting energy expenditure (~basal metabolism) was measured between 4:00 and 6:00 P.M. At 6:00 P.M., we gave a 1-g meal of either control or high-fat diet to the mice and computed the increase in energy expenditure in response to feeding (metabolism and respiratory quotient).

Leptin sensitivity. To determine leptin sensitivity, we administered recombinant mouse leptin (40 $\mu g \cdot day^{-1} \cdot mouse^{-1}$ i.p.; R&D Systems) during 4 days (at 5:00 P.M.) and evaluated food intake and/or weight loss. Food intake during 5 days before leptin injection was considered as the basal consumption (100%). An independent group of fasted animals was killed 45 min after the first leptin bolus for hypothalamus collection, protein extraction, and Western blotting quantification of pSTAT3 and STAT3.

SSR240612 chronic administration. ALZET micro-osmotic pumps (Alza, Palo Alto, CA), set to deliver for 28 days either SSR240612 (3 $mg \cdot kg^{-1}$ body wt $\cdot$ day^{-1}; Sanofi-Aventis, Paris, France) or vehicle (2% DMSO), were subcutaneously implanted in anesthetized C56Bl/6 mice under sterile conditions. Seven days after the implant, mice were submitted to high-fat diet for 3 weeks. Body weight and food intake were assessed weekly in the period of 1 month before and after the implant. At the end of the treatment, pumps were checked for their contents, and animals with filled ones were not considered in the data analysis.

Statistical analysis. All values were expressed as means ± SE. Statistical analyses were carried out using two-tailed Student's unpaired t test to compare two independent groups or ANOVA followed by Bonferroni's test to compare more than two. Significance was rejected at $P > 0.05$.

RESULTS

$B_1^{-/-}$ mice are lean. To assess possible contributions of the kinin B_1 receptor to the regulation of satiety and adiposity in mice, we analyzed body weight, energy intake, and adipose mass in standard or high-fat diet–fed $B_1^{-/-}$ and wild-type animals. Although no differences were found in the body weight (Fig. 1*A*) and energy intake (Fig. 1*B*) between wild-type and $B_1^{-/-}$ mice under control diet, a drastic reduction of fat mass was observed in the knockout animals under this condition (Fig. 1*C* and *D*). Lean mass, however, was increased in these mice, accounting for their normal body weight when compared to wild type (Fig. 1*C*). On the other hand, under high-fat diet, $B_1^{-/-}$ mice exhibited impaired energy intake (Fig. 1*B*) and were remarkably refractory to the effect of the diet on body weight gain (Fig. 1*A*). In addition, total adiposity and fat accumulation by adipocytes were dramatically reduced in $B_1^{-/-}$ mice under both diets (Fig. 1*E* and *F*), whereas the number of fat cells in the gonadal depots was only decreased in $B_1^{-/-}$ mice under standard diet (Fig. 1*G*).

Obesity is normally associated with insulin resistance and glucose intolerance. Thus, we investigated these parameters in wild-type and $B_1^{-/-}$ mice under standard or high-fat diet. In agreement, $B_1^{-/-}$ mice presented improved glucose tolerance and reduced insulin levels in both diets in comparison with the wild type (online

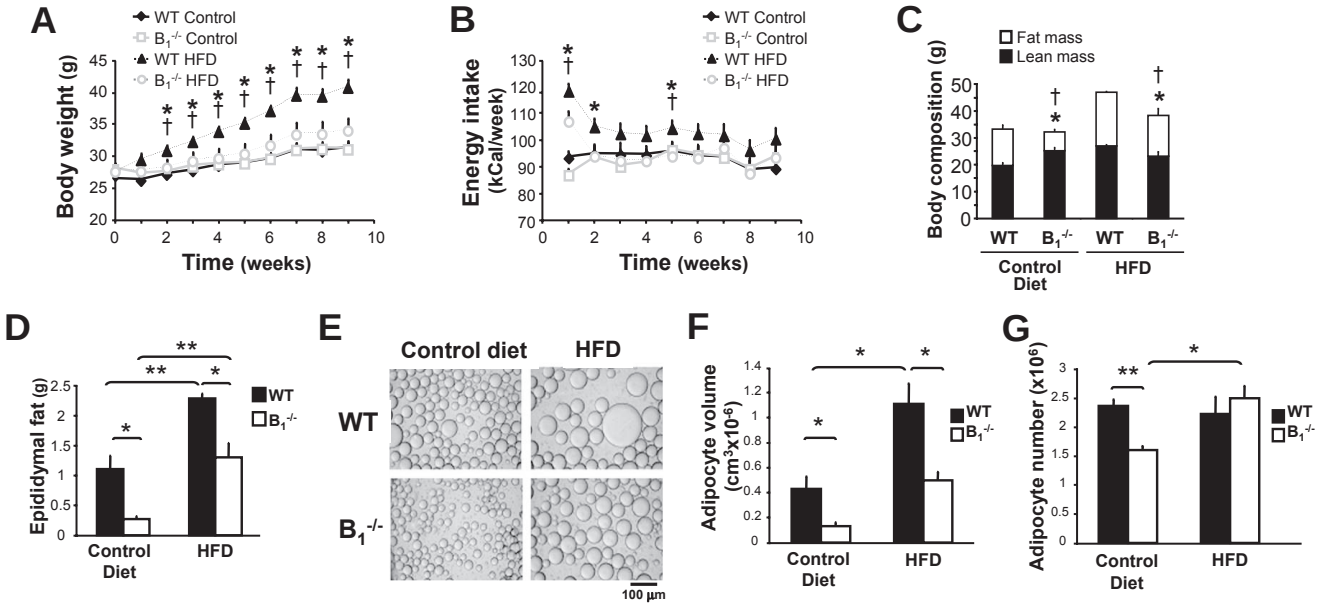

FIG. 1. Reduced fat mass in $B_1^{-/-}$ mice. Twelve-week-old wild-type and $B_1^{-/-}$ mice were submitted to standard (control) or high-fat diet (HFD) for 9 weeks and body weight (*A*), energy intake (*B*), total fat mass (*C*), epididymal fat weight (*D*), isolated adipocyte morphology (*E*), and average adipocyte volume (*F*) and number (*G*) were measured. ♦, wild type under control diet; □, $B_1^{-/-}$ under control diet; ▲, wild type under HFD; ○, $B_1^{-/-}$ under HFD. Results are means ± SE. *A* and *B*: *$P < 0.05$ $B_1^{-/-}$ HFD versus wild-type HFD. †$P < 0.05$ wild-type control diet versus wild-type HFD; $n = 11$ per group. *C*: *$P < 0.05$ $B_1^{-/-}$ fat mass versus wild-type fat mass. †$P < 0.05$ $B_1^{-/-}$ lean mass versus wild-type lean mass; $n = 5$ per group. *D*, *F*, and *G*: *$P < 0.05$. **$P < 0.01$ between indicated groups; $n = 6$ per group. Microscopy images are representative examples of six animals per group.

appendix), confirming our previous data showing increased insulin sensitivity in these mice (21).

Next, we measured serum leptin levels (Fig. 2*A*) and the expression of leptin mRNA (Fig. 2*B*) and protein (Fig. 2*C*) in the epididymal white adipose tissue of wild-type and $B_1^{-/-}$ mice. In all cases, we observed a significant decrease in leptin content in $B_1^{-/-}$ mice when compared with wild-type mice. Notably, high-fat diet did not lead to an increase of leptin levels in $B_1^{-/-}$ mice.

Taken together, these results demonstrate that $B_1^{-/-}$ mice have reduced adiposity and are strongly resistant to diet-induced hyperleptinemia and body weight gain.

Improved energy expenditure with spontaneous activity and lipid oxidation in $B_1^{-/-}$ mice under high-fat diet. Analysis of feed efficiency (body weight gain/energy consumed) (Fig. 3*A*) revealed that inhibition of weight gain observed in $B_1^{-/-}$ mice under high-fat diet could not be totally explained by decreased energy intake (Fig. 1*B*). Therefore, we quantified the components of total energy expenditure under normal and high-fat feeding in $B_1^{-/-}$ and wild-type mice. No differences in resting energy expenditure, increase in energy expenditure in response to feeding, or differences in respiratory quotient (respiratory quotient, VCO_2/VO_2) in the postabsorptive state (4 h before meal and 8–12 h after meal) were observed between $B_1^{-/-}$ and wild-type mice (Table 1). Under standard diet, respiratory quotient levels after feeding were >1.0 in both groups, suggesting that de novo lipogenesis in response to carbohydrate ingestion is occurring in these animals at a similar extent. In contrast, under high-fat diet, $B_1^{-/-}$ mice exhibited a meal-induced increase in respiratory quotient that lasted 4 h less than in wild-type mice (Fig. 3*B* and *C*), indicating that postprandial inhibition of lipid oxidation is significantly lower in these animals. In addition, $B_1^{-/-}$ mice under high-fat regimen expended significantly more energy with activity (+46.6%, $P < 0.002$; Table 1). Together, these data suggest that $B_1^{-/-}$ mice can resist high-fat diet–induced weight gain in part due to increased energy expenditure with spontaneous activity and decreased inhibition of lipid oxidation in response to a fat meal.

Hypersensitivity to leptin in $B_1^{-/-}$ mice. Together, hypoleptinemia, protection against high-fat diet–induced hyperleptinemia, elevated energy expenditure, and reduced food intake under high-fat diet suggest increased leptin responsiveness in $B_1^{-/-}$ mice. To test this hypothesis, we administered exogenous leptin to $B_1^{-/-}$ and wild-type mice and quantified daily food intake and phosphorylation of STAT3, a downstream component of the Ob-Rb signaling pathway, in the hypothalamus of these animals. A stronger inhibitory effect of exogenous leptin on food intake in $B_1^{-/-}$ mice was observed when compared with wild-type mice (Fig. 4*A*). Furthermore, the levels of phosphorylated STAT3 after leptin injection were significantly higher in the hypothalamus of $B_1^{-/-}$ in comparison with wild-type mice (Fig. 4*B*). In addition, we measured the expression of Ob-Rb mRNA in hypothalamus of $B_1^{-/-}$ and wild-type mice and found upregulation of this transcript in $B_1^{-/-}$ (Fig. 4*C*). Moreover, the expression of SOCS3, a potent inhibitor of the Ob-Rb signaling, was decreased in hypothalamus of $B_1^{-/-}$ mice (Fig. 4*D*). These data suggest that Ob-Rb signaling is facilitated in $B_1^{-/-}$ mice, which could contribute to the inhibition of food intake and enhanced energy expenditure in these animals under high-fat diet. Another aspect of leptin sensitivity, the passage of leptin through the blood-brain barrier, was addressed in $B_1^{-/-}$ and wild-type mice by measuring brain accumulation of intravenously injected [^{125}I]-labeled leptin. Although $B_1^{-/-}$ mice had a tendency to an increased leptin passage to the brain, no significant differences were observed in relation to the wild-type mice (data not shown).

We also assessed kinin B_1 receptor mRNA expression in the hypothalamus of wild-type mice after 9 weeks of high-fat

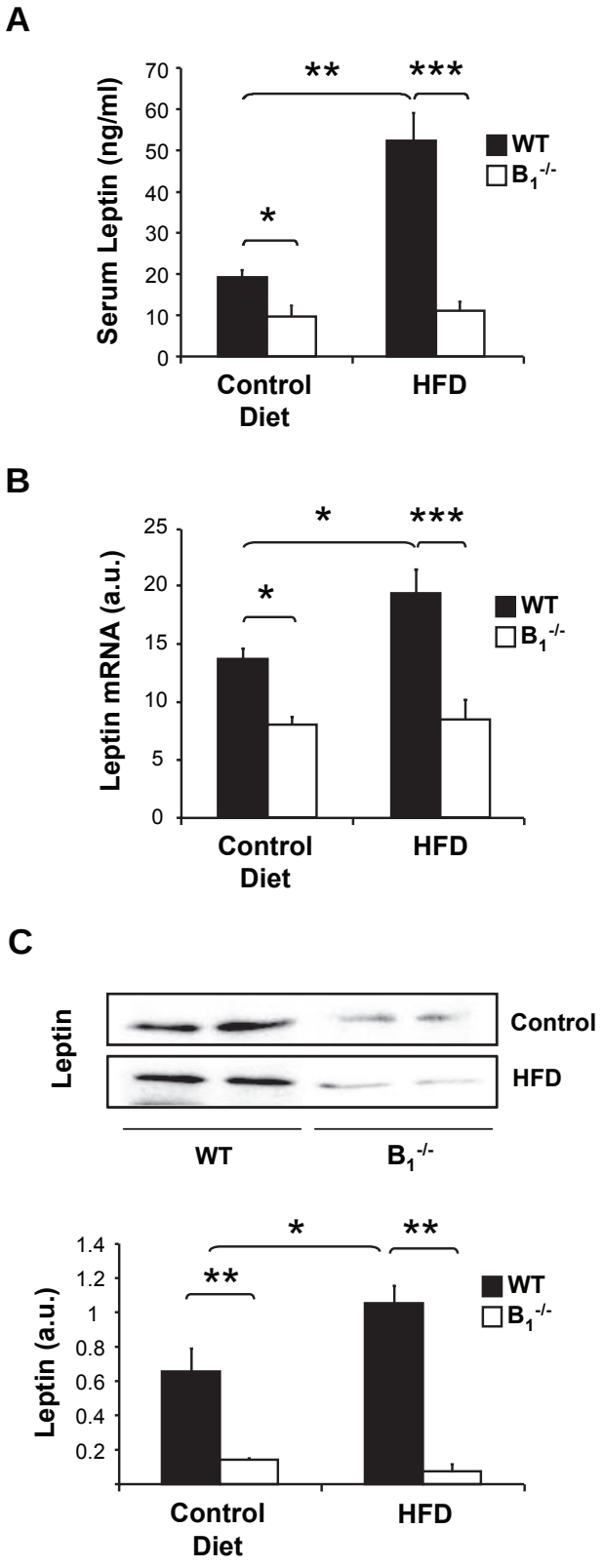

FIG. 2. Decreased leptin levels in $B_1^{-/-}$ mice. *A*: Serum leptin concentration was determined in 20 1-week-old wild-type and $B_1^{-/-}$ mice after 9 weeks under standard diet (control) and high-fat diet (HFD). *B* and *C*: Leptin mRNA and protein were quantified in the epididymal white adipose tissue of these mice, respectively. Values are means ± SE of six animals per group. Protein expression is representative of two independent pools of three animals per group. Quantification of the bands was also assessed using the software ImageJ for optical density analysis. *$P < 0.05$; **$P < 0.01$; ***$P < 0.001$ between indicated groups. a.u., arbitrary units.

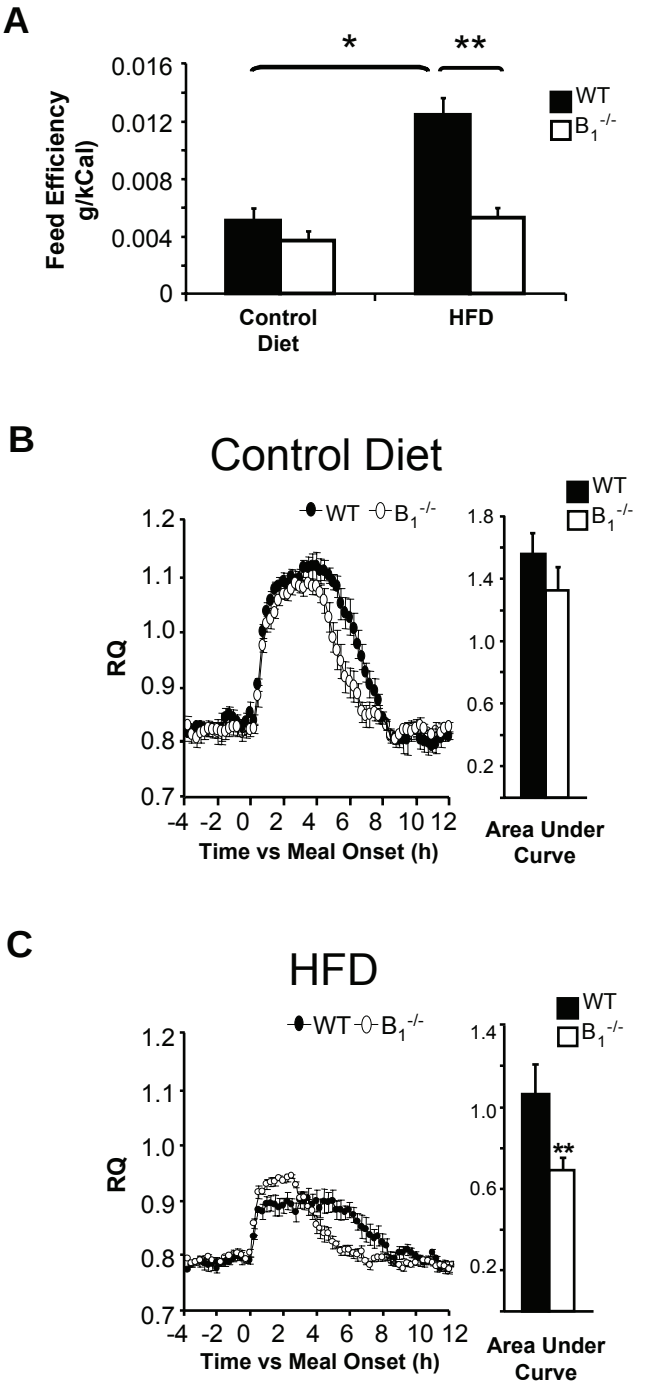

FIG. 3. Improved energy expenditure in $B_1^{-/-}$ mice. Twelve-week-old wild-type and $B_1^{-/-}$ mice were submitted to 9 weeks of standard diet (control) and high-fat diet (HFD). *A*: Feed efficiency (body weight gain/energy consumed) was calculated during this period ($n = 11$ per group). Next, mice were housed in a calorimeter for 8 h without access to food, and 1 g pellet of the corresponding diet was introduced into the metabolic cage (time 0 on the *x*-axis, 6:00 P.M. clock time) for measurement of the meal-induced changes in respiratory quotient (RQ) under standard diet (*B*) and HFD (*C*). ●, wild-type mice; ○, $B_1^{-/-}$ mice. Calorimetry results show means ± SE of five animals per group. *$P < 0.05$; **$P < 0.01$ between indicated groups.

regimen. Interestingly, we found higher levels of this mRNA in high-fat diet–fed mice, in comparison with animals under standard diet (Fig. 4*E*). Taken together, these results indicate that the kinin B_1 receptor may participate in the mechanism of leptin resistance in obesity.

TABLE 1
Components of the total energy expenditure in $B_1^{-/-}$ mice

	Control diet		High-fat diet	
	Wild type	$B_1^{-/-}$	Wild type	$B_1^{-/-}$
Resting EE (kCal · day^{-1} · kg^{-1})	2.33 ± 0.14	2.70 ± 0.31	3.26 ± 0.23	3.16 ± 0.08
Postabsorptive RQ	0.868 ± 0.012	0.853 ± 0.014	0.784 ± 0.005*	0.780 ± 0.006*
Food-induced increase in EE (kCal)	1.76 ± 0.2	1.77 ± 0.8	0.31 ± 0.3*	0.45 ± 0.4*
EE with activity (kCal · day^{-1} · kg^{-1})	123.6 ± 4.1	105.0 ± 16.3	37.9 ± 1.2*	55.7 ± 5.5*†

Data are means ± SE of Q5 animals per group. Twelve-week-old wild-type and $B_1^{-/-}$ mice were submitted to standard diet (control) and high-fat diet for 9 weeks. *$P < 0.05$ high-fat diet vs. control diet. †$P < 0.05$ wild-type vs. $B_1^{-/-}$. EE, energy expenditure; RQ, respiratory quotient.

Kinin B_1 receptor deficiency does not protect *ob/ob* mice from obesity. To estimate the importance of leptin hypersensitivity to the phenotype of resistance to obesity in $B_1^{-/-}$ mice, we generated mice lacking both B_1 receptor and leptin (*ob/ob*-$B_1^{-/-}$). In comparison with *ob/ob* mice, *ob/ob*-$B_1^{-/-}$ had no differences in body weight (Fig. 5*A*) and exhibited no significant morphological alterations, except for mildly heavier heart and kidney (data not shown). We also did not observe differences between the *ob/ob* and the *ob/ob*-$B_1^{-/-}$ mice on daily food ingestion (Fig. 5*B*) and on some parameters of adiposity in these animals, including epididymal fat content (Fig. 5*C*) and adipocyte volume (Fig. 5*D* and *E*) and number (Fig. 5*F*). These data suggest that leanness associated with kinin B_1 receptor deficiency may be dependent on the leptin signaling.

Leptin is more effective to promote weight loss in *ob/ob*-$B_1^{-/-}$ mice. Next, we performed daily injections of exogenous leptin to *ob/ob*-$B_1^{-/-}$ and *ob/ob* mice and measured food intake, body weight, and feed efficiency. Interestingly, we found that the first dose of leptin was more efficient to inhibit food intake (Fig. 6*A*) and to promote weight loss (Fig. 6*B*) in *ob/ob*-$B_1^{-/-}$ mice in comparison with *ob/ob*. In addition, *ob/ob*-$B_1^{-/-}$ presented a significant reduction of feed efficiency on the first day of leptin administration (Fig. 6*C*), indicating that weight loss in this condition is not exclusively due to inhibition of food intake. To corroborate these data, we quantified leptin-

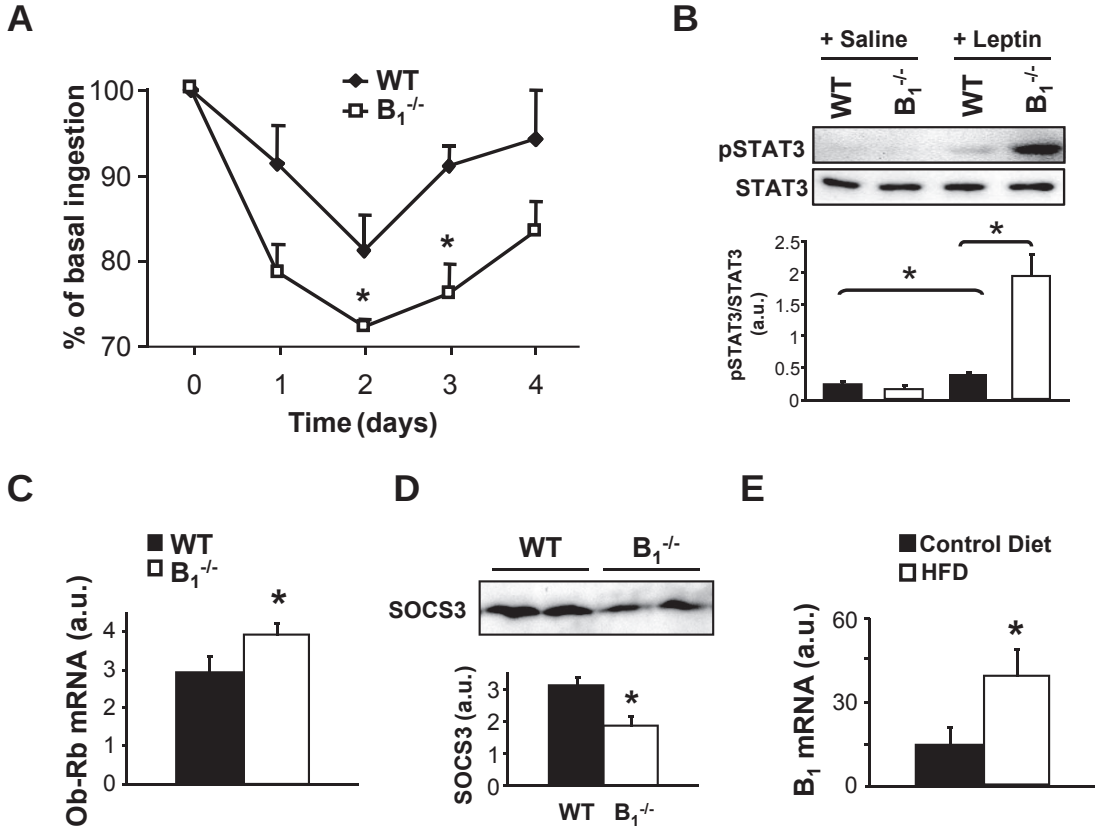

FIG. 4. Increased leptin sensitivity in $B_1^{-/-}$ mice. *A*: Inhibition of food intake of wild-type and $B_1^{-/-}$ mice under standard diet by administration of exogenous murine leptin (40 μg · day^{-1} · mouse^{-1} i.p.). Values represent percent change of the basal food ingestion collected during 5 days before leptin injections. ♦, wild-type mice; □, $B_1^{-/-}$ mice. *B*: STAT3 phosphorylation in the hypothalamus of overnight fasted wild-type (■) and $B_1^{-/-}$ (□) mice, 45 min after intraprotoneal injection of 40 μg leptin or saline. *C* and *D*: Expression of Ob-Rb mRNA and SOCS3 in the hypothalamus of overnight fasted wild-type and $B_1^{-/-}$ mice under standard diet was assessed by real-time PCR and Western blot, respectively. *E*: B_1 mRNA expression in the hypothalamus of C57Bl/6 mice after 9 weeks of control or high-fat diet (HFD). STAT3 and SOCS3 expression is representative of two independent protein pools of five animals per group. Histograms below these panels represent the quantification of the bands using the software ImageJ for optical density analysis. All other results show means ± SE of at least five animals per group. Twelve- to 14-week-old animals were used in these experiments. *$P < 0.05$. a.u., arbitrary units.

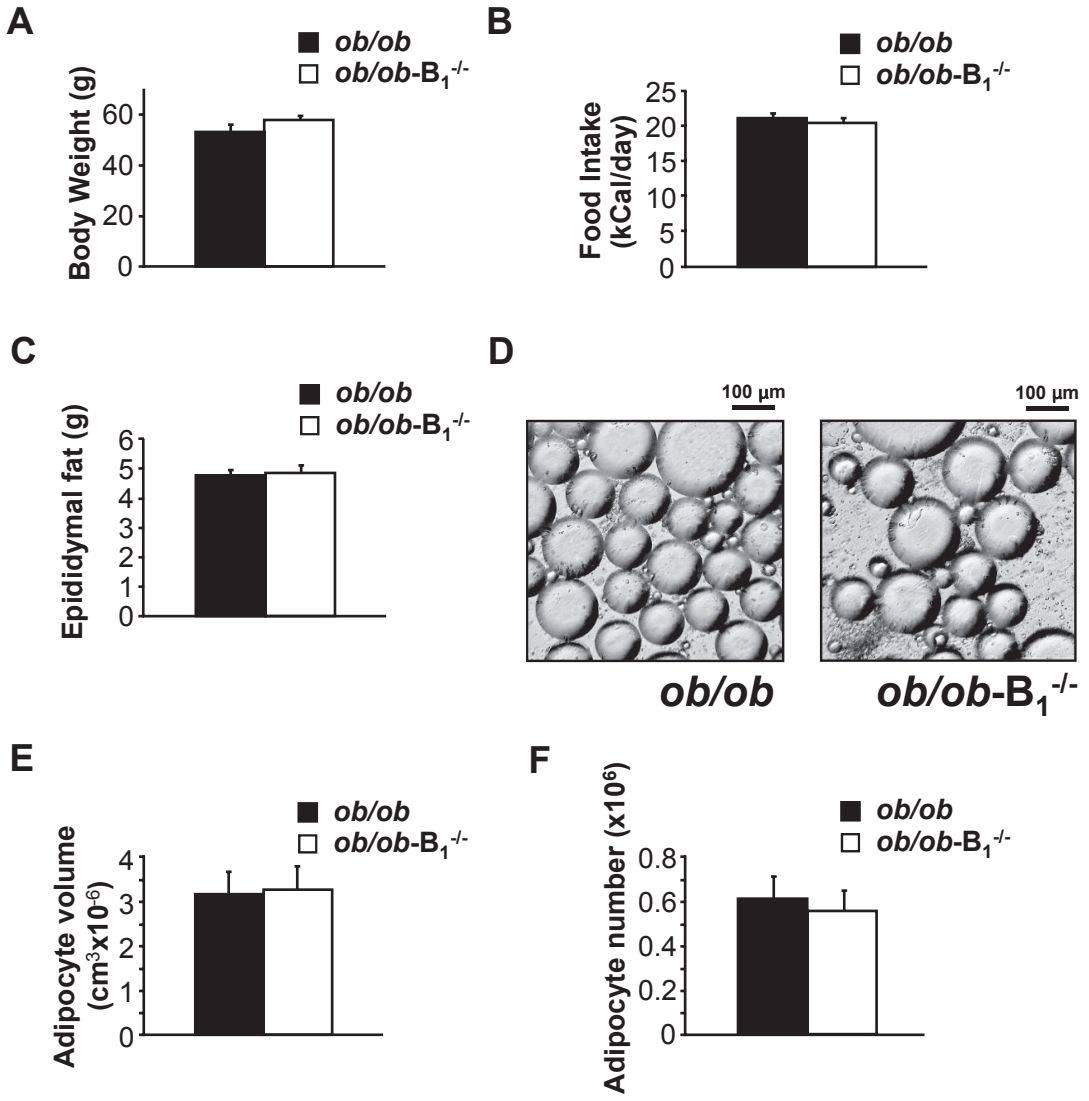

FIG. 5. Adiposity in *ob/ob*-$B_1^{-/-}$ mice. *A*: Twelve-week-old male *ob/ob* and *ob/ob*-$B_1^{-/-}$ mice were weighed. *B*: The daily food intake was measured during 2 weeks. Results represent means ± SE of the average daily values of body weight or food ingestion during this period. Mice were then killed, and the epididymal fat content was extracted, weighed (*C*), and used for adipocyte isolation. *D*: Optical microscopy images of the isolated adipocytes are represented. The quantifications of the average adipocyte volume and number are shown in *E* and *F*, respectively. All data show means ± SE of four animals per group.

stimulated STAT3 phosphorylation in the hypothalamus of *ob/ob*-$B_1^{-/-}$ and *ob/ob* mice. As expected, *ob/ob*-$B_1^{-/-}$ showed increased levels of phosphorylated STAT3 after leptin induction (Fig. 6*D*). These results bring forth the hypothesis that the kinin B_1 receptor may play a role in energy homeostasis through the modulation of leptin responsiveness.

The kinin B_1 receptor antagonist SSR240612 inhibits high-fat diet–induced weight gain, energy intake, and feed efficiency in mice. To validate our findings using knockout models and to confirm the pathophysiological function of the kinin B_1 receptor in obesity, we pharmacologically blocked this receptor in mice for 28 days by chronic administration of the nonpeptide orally active B_1 receptor antagonist SSR240612 (3 mg · kg^{-1} body wt · day^{-1}) and measured body weight gain and food intake weekly during this period. In the 1st week, mice were fed with a standard diet and showed no significant differences in body weight (Fig. 7*A*) and energy intake (Fig. 7*B*) between the groups receiving SSR240612 or the vehicle. However, after being submitted to a high-fat diet, mice receiving SSR240612, in comparison with the control mice, showed a clear inhibition in body weight gain (Fig. 7*A*) and energy intake (Fig. 7*B*). In addition, feed efficiency under high-fat diet was significantly decreased in mice treated with SSR240612 (Fig. 7*C*). Thus, these data show ample evidence to support the hypothesis that kinin B_1 receptor blockade protects mice from weight gain.

DISCUSSION

Although significant progress has been made in the identification of genes contributing to metabolic diseases, many pieces of the puzzle are still missing. Our data indicate that one member of the kallikrein-kinin system, the kinin B_1 receptor, may be an important part in this scenario because it seems to play a significant role in the regulation of food intake and energy expenditure exerted by leptin. Before this study, however, few relevant physiological roles had been attributed exclu-

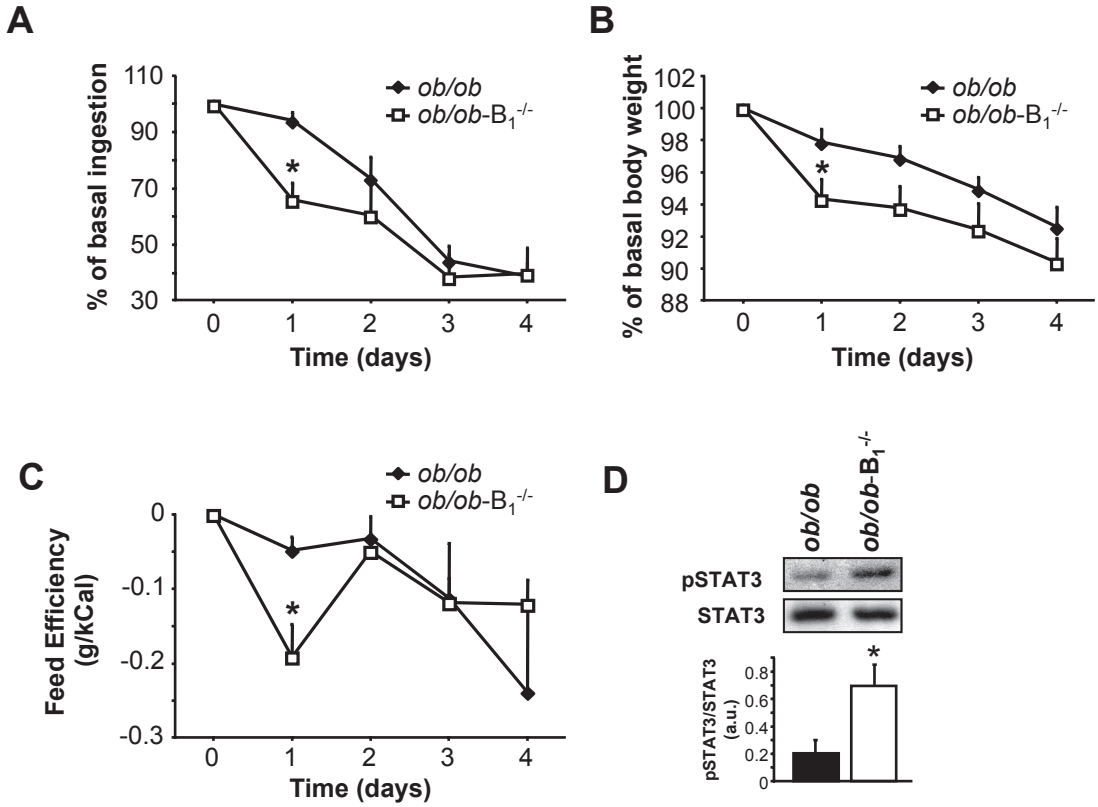

FIG. 6. Leptin sensitivity in *ob/ob*-$B_1^{-/-}$ mice. Murine leptin (40 μg · day^{-1} · $mice^{-1}$) was injected intraperitoneally in 18-week-old *ob/ob* and *ob/ob*-$B_1^{-/-}$ mice during 4 days. *A*: Food intake. *B*: Body weight. Data represent percent change to the basal values collected during 5 days before leptin injections. ♦, *ob/ob* mice; □, *ob/ob*-$B_1^{-/-}$ mice. *C*: Feed efficiency (body weight gain/energy consumed) was also calculated during this period. Results are means ± SE of four animals per group. *$P < 0.05$. *D*: STAT3 phosphorylation was quantified in the hypothalamus of *ob/ob* (■) and *ob/ob*-$B_1^{-/-}$ (□) 45 min after a leptin bolus (40 μg). The data are representative of two independent protein pools of three animals per group. The histogram represents the quantification of the bands using the software ImageJ for optical density analysis.

sively to the activation of the kinin B_1 receptor. Moreover, in most physiological processes in which the expression of B_1 receptors was observed, the kinin B_1 receptor was considered a secondary player, serving as an alternative in case of B_2 receptor deficiency (14,27). However, recent results from our group showed that kinin B_1 receptor activation modulates leptin homeostasis (24). In addition, here we present sufficient evidence to support the idea that kinin B_1 receptors are in fact participating in essential metabolic processes.

The kinin B_1 receptor, like many inflammatory molecules, including tumor necrosis factor-α (TNF-α) (28) and LPS (29), may act through redundant pathways to display both its inflammatory and metabolic actions. Similarly, leptin has inflammatory actions and strongly controls energy homeostasis (6), mainly by regulating satiety and potentiating many components of the total energy expenditure, including the energy expended with physical activity (8) and with fat oxidation (7,30). According to our data, leptin may be a key mediator of the metabolic actions of the kinin B_1 receptor. Firstly, despite the gain of fat tissue after the high-fat regimen, $B_1^{-/-}$ mice are completely protected against high-fat diet–induced hyperleptinemia, suggesting that leptin is not correlated with adiposity levels in these mice. Furthermore, *ob/ob*-$B_1^{-/-}$ and *ob/ob* mice are similarly obese, indicating that leptin may be necessary for the kinin B_1 receptor to affect food intake and energy expenditure. Finally, B_1 receptor deficiency increases leptin responsiveness and exacerbates the effects of exogenous leptin on weight loss in *ob/ob* mice.

Although the precise mechanism underlying the interaction between the kinin B_1 receptor and the leptin signaling pathway is still unclear, accumulating evidence showing the presence of this receptor and other components of the kallikrein-kinin system in the hypothalamus of rodents and humans (23,31,32) lead us to the hypothesis that the modulation of leptin sensitivity by the B_1 receptor may involve a central phenomenon. In agreement, we observed more pronounced effects of exogenous leptin administration on food intake inhibition in $B_1^{-/-}$ mice. These experiments, based on previously described protocols (8,33,34), reflect observations obtained from daily injections of high doses of leptin and may therefore represent supraphysiological effects. Nevertheless, increased central leptin sensitivity in $B_1^{-/-}$ mice is corroborated by increased leptin-induced STAT3 phosphorylation, upregulation of Ob-Rb mRNA, and reduced levels of SOCS3 under basal conditions in the hypothalamus of these animals.

SOCS3 is a member of a family of cytokine-inducible suppressors of cytokine signaling that particularly inhibits leptin signal transduction (13). In mouse embryonic fibroblasts, TNF-α–dependent activation of the nuclear factor-κB (NF-κB) is able to induce SOCS3 expression and, consequently, elicit negative effects on STAT signaling (35). As an inflammatory mediator, the kinin B_1 receptor can also activate NF-κB (36). Therefore, a possible mechanism to explain the downregulation of SOCS3 in $B_1^{-/-}$ mice would be a reduction of B_1 receptor–induced NF-κB activity in Ob-Rb neurons. Moreover, B_1 receptor defi-

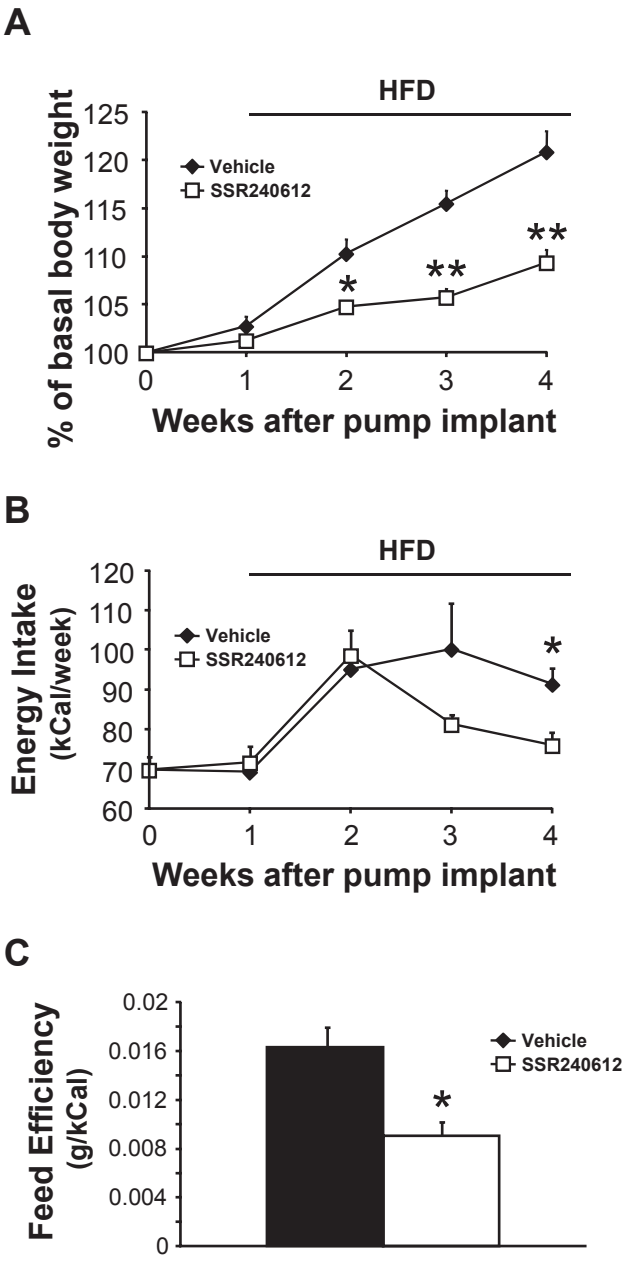

FIG. 7. SSR240612 reduces body weight gain and food intake in mice under high-fat diet (HFD). *A*: Body weight relative to basal. *B*: Energy intake. ♦, vehicle; □, SSR240612. Parameters were measured weekly in 12-week-old mice receiving either SSR240612 (3 mg · kg^{-1} body wt · day^{-1}) (n = 4) or vehicle (n = 5) for 28 days. One week after the pump implant, mice were submitted to HFD. *C*: Feed efficiency during the HFD period was also calculated in these animals. *P < 0.05; **P < 0.01.

ciency could lead to an understimulation of the hypothalamic cytokine signaling due to a decrease in cytokine production. Studies have shown that kinins can induce TNF-α and interleukin (IL)-1 release from macrophages (37) and IL-6 expression by astrocytes (38).

Obesity has also been linked to increased inflammation and macrophage infiltration in the adipose tissue (39). It is postulated that cytokines produced by the adipose tissue, which include leptin, TNF-α, and some ILs, are pivotal factors in the control of metabolism. In previous studies, B_1 receptor deficiency has been associated with hypoalgesia and reduced inflammatory response (18,19). It was demonstrated that the hypotensive effect of LPS was blunted in $B_1^{-/-}$ mice and the infiltration of polymorphonuclear leukocytes into tissues. The contributions of the kinin B_1 receptor to the inflammatory status of the adipose tissue are, however, not clear. Thus, future experiments may be proposed to understand how the absence of B_1 receptors in the adipose tissue could modulate inflammatory mediators to participate in the phenotypes observed in $B_1^{-/-}$ mice.

On the other hand, the regulation of the energy balance by kinin B_1 receptor may not be related exclusively to activation by kinins. Recently, Lai et al. (40) presented strong evidence that kinins are not the only agonists that bind to kinin receptors. They showed that the endogenous opioid peptide dynorphin A promotes neuropathic pain through the activation of both kinin B_1 and B_2 receptors. In the early 80s, 3 years after dynorphins were identified as opioid regulators of pain and stress responses, these peptides were described as potent inducers of food intake (41,42). However, in 2 decades, modest advances have been made to provide a solid understanding of the mechanisms mediating the regulation of energy balance by dynorphin. Nevertheless, evidence showing that dynorphin neurons in the arcuate nucleus are leptin responsive (43) and that the effect of κ-opioid agonists on food intake cannot be reproduced in *ob/ob* mice (44) indicates that leptin may be required for this event to happen. Thus, we speculate that dynorphin may act through the kinin B_1 receptor to control food intake via modulation of the leptin signaling.

In general, protection against obesity is a common feature of mice with leptin hypersensitivity. SOCS3 gene disruption in the brain, for instance, is able to increase leptin sensitivity and promote resistance to high-fat diet–induced weight gain and hyperleptinemia (33). Moreover, the neuron-specific tyrosine phosphatase protein-tyrosine phosphatase 1B (PTP1B) knockout mice are over-responsive to the effects of exogenous leptin and show reduced adiposity and elevated energy expenditure with activity (34). Thus, targeting SOCS3 and targeting PTP1B are currently considered attractive strategies to fight obesity and diabetes.

Similarly, CB_1 cannabinoid receptor knockout mice (45,46) are more sensitive to intracerebroventricular injections of leptin, have decreased body weight, and are slightly hypophagic. In addition, they are resistant to high-fat diet–induced weight gain due to an increase in energy expenditure. Based on this animal model, researchers evidenced a strong anti-obesity effect of the CB_1 receptor antagonist SR141716A (46), and currently, under the name of rimonabant, this drug has been considered the most efficient clinical therapy to treat human obesity (47). Therefore, based on the presented data, here we suggest the orally active kinin B_1 receptor antagonist SSR240612 as a potential tool to prevent obesity. Although several experiments still must be addressed to verify the clinical relevance of this drug, we believe that our results and the grown body of evidence showing limited expression of B_1 receptors under physiological conditions (14) may render the kinin B_1 receptor an interesting potential target to treat obesity with low risks of severe side effects.

In summary, we have presented in this study consistent data indicating that functional deficiency of the kinin B_1 receptor can protect mice from obesity through a mechanism that may involve leptin signaling.

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