

# Lack of Responses to a $\beta_3$ -Adrenergic Agonist in Lipoatrophic A-ZIP/F-1 mice

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**Stimulation of  $\beta_3$ -adrenergic receptors increases metabolic rate via lipolysis in white adipose tissue (WAT) and thermogenesis in brown adipose tissue (BAT). Other acute effects include decreased gastrointestinal motility and food intake and increased insulin secretion. Chronic treatment with a  $\beta_3$  agonist ameliorates diabetes and obesity in rodents. We studied the effects of  $\beta_3$  stimulation in A-ZIP/F-1 mice, which have virtually no WAT, a reduced amount of BAT, severe insulin resistance, and diabetes. In contrast with wild-type mice, treatment of A-ZIP/F-1 mice with CL316243, a  $\beta_3$ -adrenergic agonist, did not increase  $O_2$  consumption. A single dose of CL316243 produced a 2-fold increase in serum free fatty acids, a 53-fold increase in insulin, and a 2.4-fold decrease in glucose levels in wild-type mice but no change in A-ZIP/F-1 animals. The A-ZIP/F-1 mice also did not show reduced gastrointestinal motility or 24-h food intake during  $\beta_3$  stimulation. Chronic administration of CL316243 to the A-ZIP/F-1 mice did not improve their thermogenesis, hyperglycemia, or hyperinsulinemia. Thus, all of the  $\beta_3$  effects studied were absent in the lipoatrophic A-ZIP/F-1 mice, including the effects on nonadipose tissues. From these results, we suggest that all of the effects of  $\beta_3$  agonists are initiated at the adipocyte with the nonadipose effects being secondary events presumably mediated by signals from adipose tissue. *Diabetes* 49:1910–1916, 2000**

**T**he  $\beta_3$ -adrenergic receptor is a G protein-coupled receptor found predominantly on adipocytes (1–3).  $\beta_3$ -Adrenergic receptor activity and mRNA have also been reported in the smooth muscle of the gastrointestinal tract and in other sites (2,4,5).  $\beta_3$ -Adrenergic stimulation causes lipolysis in white adipose tissue (WAT) and thermogenesis in brown adipose tissue (BAT). Chronic administration of a  $\beta_3$ -adrenergic agonist leads to decreased fat mass and improvement of diabetes in rodent obesity models (6,7). Human  $\beta_3$  research has been hampered by the lack of a sufficiently selective drug (8), but in one study,  $\beta_3$ -agonist treatment increased nonoxidative glucose disposal and fractional fat oxidation (9). In rodents, acute  $\beta_3$ -ago-

nist treatment has effects besides stimulating lipolysis and thermogenesis, such as inhibiting gastrointestinal motility and food intake and stimulating insulin secretion. The mechanisms underlying these effects are not well understood.

Several transgenic mouse lines with reduced amounts of adipose tissue have been developed (10–13). In the A-ZIP/F-1 mouse, adipose tissue ablation was achieved by adipose-selective expression of a dominant negative protein to certain B-ZIP transcription factors. The A-ZIP/F-1 mice have virtually no WAT, a reduced amount of BAT, leptin deficiency, severe insulin resistance and diabetes, and an accelerated adaptation to fasting (12,14). Surgical transplantation of adipose tissue into A-ZIP/F-1 mice reverses their metabolic syndrome, which demonstrates that the lack of fat is the cause of the metabolic abnormalities (15,16).

The nearly complete lack of WAT in the A-ZIP/F-1 mice makes these animals a valuable tool for studying the biology of adipose tissue. Herein we study the role of adipose tissue in the whole-animal response to  $\beta_3$ -adrenergic agonist treatment. Interestingly, we found that all of the effects of  $\beta_3$ -agonist treatment, those affecting adipose and nonadipose tissue, are ablated in these mice.

## RESEARCH DESIGN AND METHODS

**Mice.** The A-ZIP/F-1 mice (12) were hemizygous on the FVB/N background and were maintained on a 12-h light/dark cycle (6 A.M./6 P.M.). Only female mice were used. Pellet diet (NIH-07, 5% fat by weight) and drinking water were provided ad libitum. Mice were typically housed 4 mice/cage and were studied at 22–25°C (unless noted otherwise).

**Indirect calorimetry.**  $O_2$  consumption and  $CO_2$  production were measured by using a four-chamber Oxymax system (Columbus Instruments, Columbus, OH) with one mouse/chamber and by testing transgenic mice simultaneously with littermate controls. Motor activity (total and ambulating) was determined by infrared beam interruption (Opto-Varimex mini, Columbus Instruments). Mice had free access to food and water. Resting  $O_2$  consumption was calculated as the average of the points with <6 ambulating beam breaks/min and omission of the first hour of the experiment. The respiratory exchange ratio (RER) (the ratio of  $CO_2$  produced to  $O_2$  consumed) was calculated using the same data points. Oxidation of carbohydrate produces an RER of 1.00, whereas fatty acid oxidation results in an RER of ~0.70 (17).  $O_2$  consumption data were normalized to (body weight)<sup>0.75</sup> (18,19), although mice of similar weights were used within each experiment.

The effect of the  $\beta$ -adrenergic agonists, CL316243 ( $\beta_3$ -selective [20]) and isoproterenol (nonselective  $\beta$ ), were measured as follows with each mouse serving as its own control. At ~9 A.M., mice were placed into the calorimetry chambers (prewarmed to 30°C), and baseline data were collected. After 3 h, CL316243 was injected (1 mg/kg i.p.), and, after a 1-h delay, data were collected for 2 h. When isoproterenol (0.3 mg/kg s.c.) was used, data were collected for the first hour after injection because the increase in  $O_2$  consumption disappeared within 1.5 h. These conditions were chosen to maximize the agonist effect and to minimize the experimental variability. However, similar results were observed under various conditions (time after dosing, amount of physical activity) for both CL316243 and isoproterenol.

**Chronic treatment with CL316243.** Female mice (initially 4 weeks of age,  $n = 6$ /group) were treated with saline or CL316243 (1 mg · kg<sup>-1</sup> · day<sup>-1</sup> i.p. at 11 A.M.) for 17 days. Body weight and food intake were measured daily or every

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BAT, brown adipose tissue; FFA, free fatty acid; RER, respiratory exchange ratio; Ucp1, uncoupling protein 1; WAT, white adipose tissue.

3 days, respectively. Blood was obtained on day 15, O<sub>2</sub> consumption was measured on day 17, and the mice were killed on day 18.

**Biochemical assays.** Glucose was measured using a Glucometer Elite (Bayer). Insulin was measured by radioimmunoassay (SRI-13K; Linco, St. Charles, MO), and free fatty acids (FFAs) were measured enzymatically (1383175; Boehringer Mannheim, Mannheim, Germany).

**Isolation and analysis of RNA.** RNA was extracted using TRIzol Reagent (Gibco, Grand Island, NY). Northern blots (Maximum Strength Nytran Plus; Schleicher & Schuell, Keene, NH) were hybridized using Rapid-hyb (Amersham, Amersham, U.K.) according to manufacturer's directions and were quantitated with a phosphorimager (Molecular Dynamics). The rat uncoupling protein 1 (Ucp1) probe is bp 84–1154 in GenBank M11814 (21). The human  $\beta_3$ -adrenergic receptor cDNA probe was obtained from Dr. J. McLenithan, University of Maryland School of Medicine (Baltimore, MD).

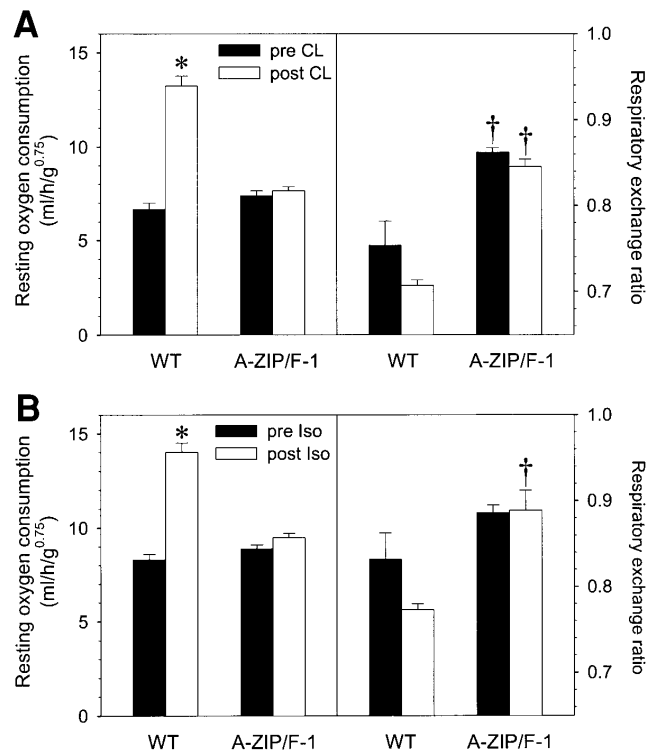
**Statistics.** Statistical significance was determined using SigmaStat by *t* test (paired or unpaired as appropriate) or analysis of variance with Tukey pairwise tests. Data are means  $\pm$  SE.

## RESULTS

**Deficient  $\beta$ -adrenergic-stimulated thermogenesis in A-ZIP/F1 mice.** We measured the acute effects of CL316243, a well-characterized highly selective  $\beta_3$ -adrenergic agonist (20,22), on wild-type and A-ZIP/F-1 mice. Acute treatment of control mice with CL316243 increased O<sub>2</sub> consumption 98% and decreased the RER, which reflects activation of thermogenesis and stimulation of lipolysis and fatty acid oxidation, respectively (Fig. 1A). A-ZIP/F-1 mice had a higher baseline RER. Unlike wild-type mice, the A-ZIP/F-1 mice showed no changes in energy expenditure or RER with CL316243 treatment. The A-ZIP/F-1 mice, unlike the wild-type animals, did not exhibit the heat-dissipating behaviors (e.g., panting, decreased ambulation, and a splayed stretched-out posture) caused by CL316243 treatment. Thus, the A-ZIP/F-1 mice are deficient in thermogenic  $\beta_3$ -adrenergic responses.

Similar results were obtained with isoproterenol, which is a nonselective  $\beta$ -adrenergic agonist (Fig. 1B). In addition to  $\beta_3$  receptors, this agonist targets  $\beta_1$  and  $\beta_2$  receptors on skeletal muscle, heart, and other tissues. In control mice, acute treatment with isoproterenol increased resting O<sub>2</sub> consumption 75% and decreased the RER. In contrast, no increase in resting O<sub>2</sub> consumption was observed in the A-ZIP/F-1 mice, although both control and A-ZIP/F-1 mice showed decreased ambulatory activity after isoproterenol. In summary, A-ZIP/F-1 mice do not increase their metabolic rate in response to a single dose of  $\beta$ -adrenergic agonist (either nonselective  $\beta$  or  $\beta_3$ -selective). These data support the hypothesis that BAT and WAT are required for the thermogenic response to  $\beta$ -adrenergic stimuli.

**Effect of chronic CL316243 treatment.** The BAT in the A-ZIP/F-1 mice appears inactive both grossly and histologically. We tested whether chronic treatment with CL316243 has a permissive effect that allowed A-ZIP/F-1 mice to respond acutely to  $\beta_3$ -adrenergic stimulation. Chronic  $\beta_3$ -agonist treatment induced histological changes in the wild-type BAT with a reduction in fat droplet size and more eosinophilic staining, which indicates an increased number of mitochondria (Fig. 2A). CL316243 treatment of A-ZIP/F-1 mice also caused the BAT to become slightly more eosinophilic, with smaller lipid droplets. However, the A-ZIP/F-1 BAT appeared to be much less active than BAT from wild-type mice both before and after  $\beta_3$ -agonist treatment. Ucp1 mRNA levels increased in both A-ZIP/F-1 and control mice, with chronic CL316243 treatment (Fig. 2B).  $\beta_3$ -Adrenergic receptor mRNA levels in BAT were similar in wild-type and A-ZIP/F-1 mice



**FIG. 1.** Effect of acute  $\beta$ -adrenergic stimulation on O<sub>2</sub> consumption. Either CL316243 (CL), a  $\beta_3$ -selective agonist (A), or isoproterenol (Iso), a nonselective  $\beta$  agonist (B), was injected, and the effects on O<sub>2</sub> consumption (left) and RER (right) were measured. Measurements were made at 30°C as described in RESEARCH DESIGN AND METHODS. Mice treated with CL316243 or saline were 8 weeks old ( $n = 5/\text{group}$ ), and those treated with isoproterenol or saline were 14 weeks old ( $n = 6/\text{group}$ ). \* $P < 0.001$ , pre- vs. posttreatment; † $P < 0.005$ , A-ZIP/F-1 vs. wild-type mice (WT). No other differences reached statistical significance.

with or without CL316243 treatment (Fig. 2B [the  $\beta_3$ -adrenergic receptor mRNA is slightly smaller in the A-ZIP/F-1 mice]). Thus, chronic CL316243 treatment shifted the A-ZIP/F-1 BAT to a more active phenotype, but it did not become as active as wild-type BAT.

With chronic CL316243 treatment, the wild-type mice showed a 16% increase in food intake with no change in body weight, reduced WAT mass, and increased accumulation of brown adipocytes within WAT (Table 1) (data not shown). In contrast, in A-ZIP/F-1 mice, CL316243 treatment did not change food intake or body weight. Also, chronic  $\beta_3$ -agonist treatment did not improve either the hyperglycemia or hyperinsulinemia of the A-ZIP/F-1 mice (Fig. 2C).

In wild-type mice, the increase in O<sub>2</sub> consumption with acute  $\beta_3$  stimulation in chronically CL316243-treated mice was greater than in saline-treated controls (20.8 vs. 17.9 ml · h<sup>-1</sup> · g<sup>-0.75</sup> in chronically treated and control mice, respectively) (Fig. 3). However, chronic CL316243 treatment did not affect acute  $\beta_3$ -stimulated O<sub>2</sub> consumption in the A-ZIP/F-1 mice. These data demonstrate that A-ZIP/F-1 BAT can respond to  $\beta$ -adrenergic stimulation. The lack of a measurable O<sub>2</sub> consumption response to  $\beta_3$ -agonist treatment in A-ZIP/F-1 mice is most likely because of the lack of WAT and the reduced amount of BAT rather than a  $\beta_3$  signaling defect.

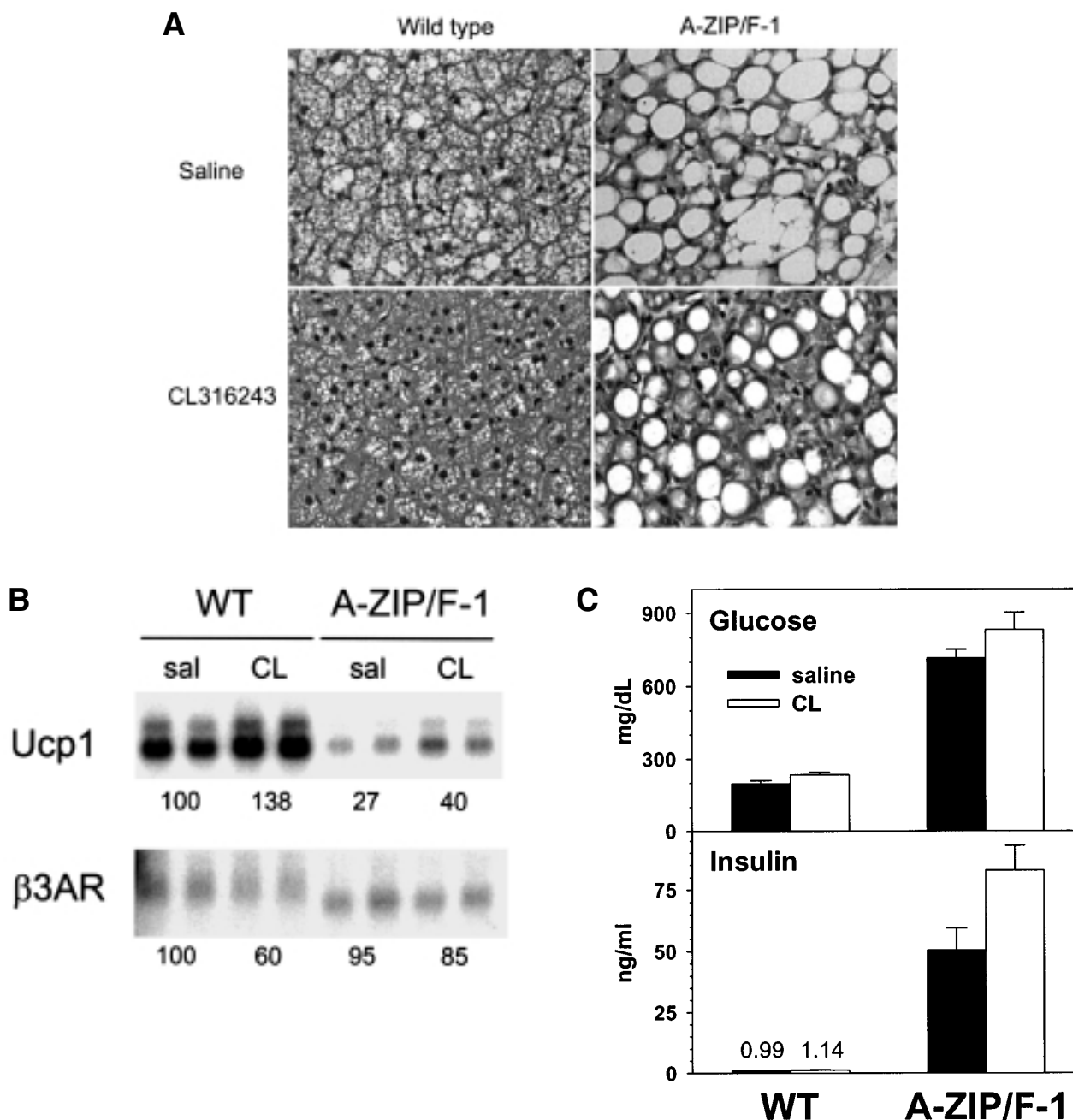


FIG. 2. Chronic treatment with CL316243 (CL). Wild-type (WT) and A-ZIP/F-1 mice (8 weeks old) were treated with CL316243 (1 mg/kg i.p., daily for 17 days) or saline (sal) as described in RESEARCH DESIGN AND METHODS. *A*: BAT histology. Wild-type and A-ZIP/F-1 mice were treated with saline or CL316243. The saline-treated A-ZIP/F-1 BAT appears to be the least active (larger fat droplets and less eosinophilia), and treatment with CL316243 produces a more active histological appearance. Original magnification 400×. *B*: BAT mRNA levels. Ucp1 and β<sub>3</sub>-adrenergic receptor (β3AR) mRNAs were measured by Northern blotting. Each lane contains RNA pooled from the BAT of three mice (10 μg/lane). Equal RNA loading was confirmed by ethidium bromide staining (data not shown). Signals were quantitated using a phosphorimager, and the averaged results are expressed in arbitrary units. *C*: Serum glucose and insulin were measured in fed morning samples (obtained before the daily dose of CL316243) on day 15 of treatment (*n* = 6/group).

**Effect of CL316243 on food intake and gastrointestinal motility.** In addition to increasing O<sub>2</sub> consumption, acute treatment with CL316243 has other effects, including inhibition of food intake (22) and of gastrointestinal motility (23). We used lipotrophic A-ZIP/F-1 mice to test the role of adipose tissue in these effects. Intestinal length was increased in the A-ZIP/F-1 mice (50.3 ± 2.0 mm in A-ZIP/F-1 mice vs. 39.1 ± 2.0 mm in controls, *n* = 6/group; *P* = 0.003) out of proportion

with any increase in body weight (28.2 ± 0.9 g in A-ZIP/F-1 mice vs. 27.0 ± 1.5 g in controls, *n* = 6/group; *P* = NS). Histologically, no gastrointestinal abnormalities were observed (data not shown). In wild-type mice, a single dose of CL316243 caused a 25% inhibition of food intake (Fig. 4A). As noted previously (12), A-ZIP/F-1 mice eat more than the wild-type mice, but in contrast with the controls, their food intake was not decreased by CL316243 administration (Fig. 4A).

TABLE 1  
Effects of chronic CL316243 administration

	Wild-type mice		A-ZIP/F-1 mice	
	Saline	CL316243	Saline	CL316243
Body weight (g)	19.7 ± 0.5	19.5 ± 0.6	18.2 ± 0.5	19.4 ± 0.4
Change in body weight (g)	0.4 ± 0.5	0.7 ± 0.8	2.6 ± 1.8	3.5 ± 3.2
Food intake (g/day)	4.2 ± 0.1	5.0 ± 0.2*	5.9 ± 0.3	6.6 ± 0.3
Liver weight (g)	1.07 ± 0.07	1.01 ± 0.04	1.52 ± 0.15	1.80 ± 0.08
Interscapular BAT (mg)	115 ± 7	113 ± 7	54 ± 3	58 ± 4
Parametrial WAT (mg)	331 ± 27	188 ± 24*	<20	<20
Retroperitoneal WAT (mg)	80 ± 13	45 ± 10*	<20	<20
Inguinal WAT (mg)	240 ± 30	194 ± 7	<20	<20

Data are means ± SE. Mice (initially 4 weeks old,  $n = 6/\text{group}$ ) were treated with saline or CL316243 (1 mg/kg) daily. Mice were killed between 10 A.M. and 1 P.M. on day 18. \* $P < 0.05$  saline vs. CL316243 within genotype.

Similarly, in contrast with the 54% inhibition of gastrointestinal motility observed in the wild-type mice, acute administration of CL316243 had no effect on gastrointestinal motility in the A-ZIP/F-1 mice (Fig. 4B). Therefore, the presence of adipose tissue is required for the  $\beta_3$ -agonist effect on both food intake and gastrointestinal motility.

**Effect of acute treatment with CL316243 on glucose and insulin levels.** Acute treatment with CL316243 stimulates insulin secretion (24). To assess the role of adipose tissue in the stimulation of insulin secretion, we measured FFA, insulin, and glucose levels 15 min after administration of CL316243 (Fig. 5). In fed wild-type mice, this treatment increased FFA and insulin levels (2- and 53-fold, respectively) and lowered glucose levels (2.4-fold, Fig. 5). In contrast, no changes were evident in these parameters in the A-ZIP/F-1 mice. Because the insulin levels in fed A-ZIP/F-1 mice were 57-fold higher than in controls and therefore may already be maximally stimulated, we repeated the experiment using mice fasted for 7 h, which had 30-fold lower baseline insulin

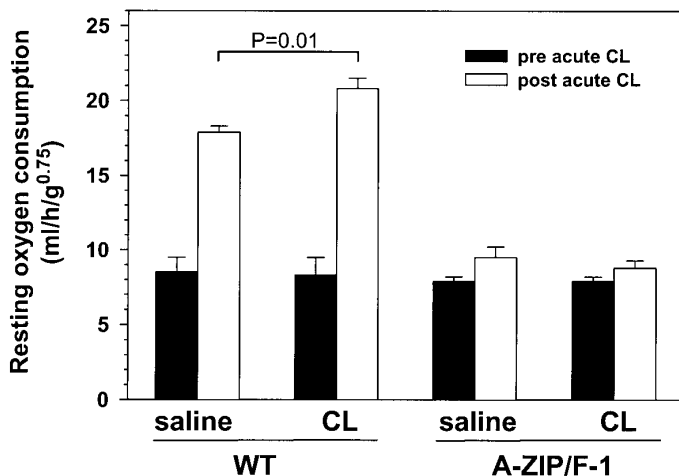


FIG. 3. Acute effect of CL316243 (CL) on  $O_2$  consumption in mice chronically treated with CL316243. Mice (initially 4 weeks old) were treated with CL316243 (1 mg/kg i.p.) daily for 16 days. The 17th day's dose was used to test the acute effects of CL316243 treatment as described in RESEARCH DESIGN AND METHODS ( $n = 4/\text{group}$ ). WT, wild-type mice.

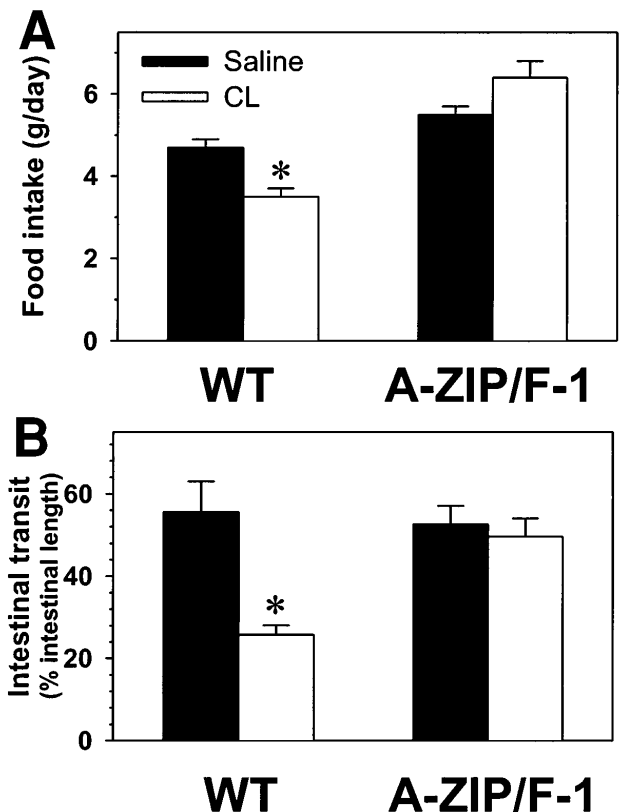


FIG. 4. Effect of a single dose of CL316243 (CL) on food intake and gastrointestinal transit. **A:** Food intake was measured in individually caged 10-week-old mice ( $n = 6/\text{group}$ ) that received daily saline injections for 3 days. On day 4, mice were injected with CL316243 (1 mg/kg i.p.) or saline, and food intake was measured over the next 24 h. CL316243 caused a significant decrease in food intake only in wild-type mice (WT) (\* $P = 0.008$ ). A-ZIP/F-1 mice ate more than wild-type mice independent of treatment ( $P = 0.02$ ). **B:** Gastrointestinal transit was measured in 17-week-old mice ( $n = 6/\text{group}$ ). Mice were fasted overnight and then injected with CL316243 (1 mg/kg i.p.) or saline. After 1 h, each mouse received 0.2 ml 2% activated charcoal in 1.2% gum arabic intragastrically and was killed 20 min later. Gastrointestinal transit is expressed as the distance from the pyloric sphincter traveled by the charcoal front, which is expressed as a percentage of the small intestine length (pyloric sphincter to ileocecal junction). \* $P = 0.036$ , saline vs. CL316243 in wild-type mice.

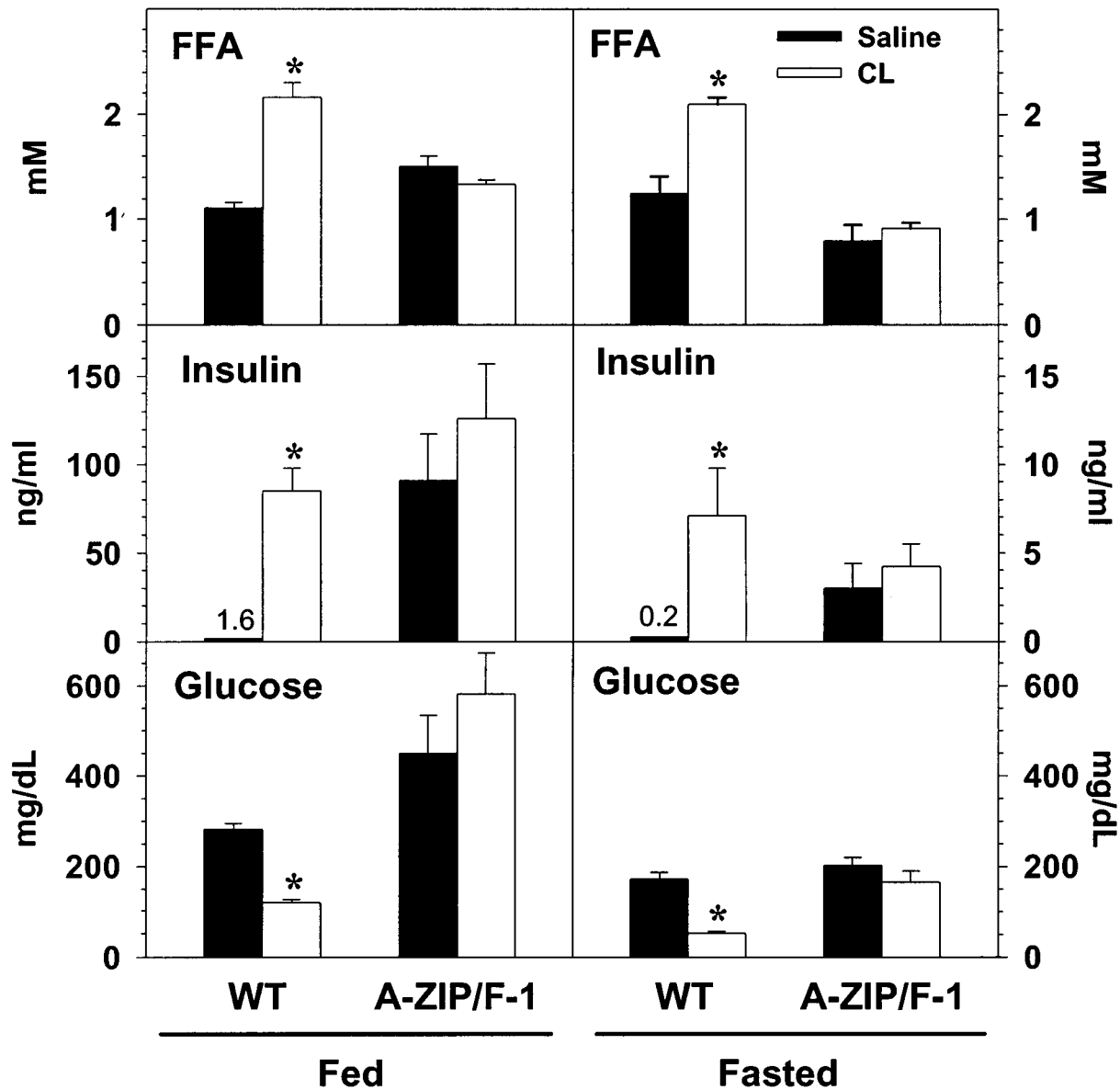


FIG. 5. Effect of acute CL316243 (CL) treatment on FFA, insulin, and glucose levels. Mice (16 weeks old) were treated with CL316243 (1 mg/kg i.p. at 3 P.M.), and 15 min later, serum for measurement of FFA, insulin, and glucose levels was obtained. Mice were fed (left) or fasted for 7 h (right) before the experiment. Note the 10-fold difference in the insulin scales between fed and fasted mice. WT, wild-type mice. \**P* < 0.03, saline vs. CL316243 in wild-type mice.

levels (Fig. 5). The fasted A-ZIP/F-1 mice did not change their insulin levels in response to acute treatment with CL316243 versus the 43-fold increase in fasted controls. Thus, the ability of the β<sub>3</sub> agonist to stimulate insulin secretion acutely requires the presence of adipose tissue.

**DISCUSSION**

**Relative role of BAT, WAT, and other tissues in the stimulation of metabolic rate by the β<sub>3</sub> agonist.** Defects in both BAT and WAT are likely to contribute to the lack of β<sub>3</sub>-stimulated O<sub>2</sub> consumption in A-ZIP/F-1 mice. Most β<sub>3</sub>-stimulated energy expenditure and heat production are thought to occur in BAT, and mice with ablated BAT show reduced β<sub>3</sub> thermogenesis (25). In A-ZIP/F-1 mice, BAT undergoes accelerated involution, so that in adults it is greatly reduced in amount and is inactive (12). In other mod-

els, diabetes is associated with inactive BAT, possibly because of a low sympathetic tone (26,27). In the A-ZIP/F-1 mice, diabetes probably contributes to BAT inactivation because the BAT inactivity is partially reversed by either chronic β<sub>3</sub> treatment or WAT transplantation. The A-ZIP/F transgene itself is another contributor to BAT inactivity because it could disrupt the normal pattern of BAT gene expression. These two causes of decreased BAT function together contribute to the decreased thermogenic response to β<sub>3</sub> stimulation in A-ZIP/F-1 mice.

However, A-ZIP/F-1 mice remain resistant to acute β<sub>3</sub> stimulation even after chronic β<sub>3</sub> treatment, which partially reverses the BAT inactivation. This indicates that defects in BAT alone do not explain the resistance to acute β<sub>3</sub> stimulation. Much of the energy burned by BAT comes from FFAs released from WAT. In A-ZIP/F-1 mice, we estimate that the

WAT mass in adult mice is reduced by ~99% (28), and total triglyceride levels are reduced by 90% (14). Thus, the lack of  $\beta_3$  metabolic response in A-ZIP/F-1 mice is also because of the lack of the WAT triglyceride fuel in addition to BAT inactivity.

In a complementary approach, Susulic and colleagues (22,29) used mice lacking  $\beta_3$ -adrenergic receptors and restored  $\beta_3$  receptors selectively in BAT alone or in both WAT and BAT. Mice lacking  $\beta_3$  receptors had no response to CL316243. Some of the response was restored by expression of  $\beta_3$  receptors in BAT alone, whereas a robust response was recreated with expression of  $\beta_3$  receptors in both WAT and BAT (22,29). Our studies ablating WAT and BAT complement the studies of Lowell and colleagues in which WAT and BAT were intact but signaling was ablated. These two approaches have yielded concordant results, which demonstrates that both BAT and WAT contribute to the metabolic response to  $\beta_3$  stimulation.

Evidence exists that tissues besides WAT and BAT contribute to thermogenesis. For example, A-ZIP/F-1 mice can rewarm themselves while waking from torpor (14). Increased muscle metabolism after treatment with a  $\beta_3$  agonist led to the suggestion that muscle is a direct  $\beta_3$  target (30). Thus, we were surprised to find no detectable increase in resting metabolic rate in A-ZIP/F-1 mice treated with isoproterenol, which is a nonselective  $\beta$  agonist that targets many tissues. In non-lipoatrophic animals, we assume that thermogenesis in muscle and viscera occurs via oxidation of fatty acids released from adipose tissue. In A-ZIP/F-1 animals, the lack of fuel limits thermogenesis at all sites.

**Role of adipose tissue in  $\beta_3$  effects on the pancreas and gastrointestinal tract.** Some reports have suggested that CL316243 directly stimulates insulin secretion from the pancreas (31). However, the increase in insulin occurs after the increase in serum FFAs (24), which suggests that insulin secretion is stimulated by a signal from adipose tissue. Support for the hypothesis that adipose  $\beta_3$  signaling is needed for insulin secretion comes from the observation that  $\beta_3$ -stimulated insulin secretion is intact in mice expressing  $\beta_3$  receptors only in adipose tissue (29). Our data showing no increase in insulin secretion in response to a  $\beta_3$  agonist in A-ZIP/F-1 mice also buttress the adipose signal-to- $\beta$ -cell paradigm. The nature of this signal is not known; it could include FFAs, glycerol, and/or peptide hormones.

Chronic  $\beta_3$ -agonist treatment causes increased insulin sensitivity in various models (9,32–34). However, chronic CL316243 administration had no effect on the diabetes phenotype of A-ZIP/F-1 mice, which suggests that the antidiabetic effects of this  $\beta_3$  agonist require adipose tissue.

In A-ZIP/F-1 mice, CL316243 did not inhibit gastrointestinal tract motility. Evidence suggesting a direct action of the  $\beta_3$  agonist on the gastrointestinal tract includes the identification of  $\beta_3$ -adrenergic receptors throughout the gastrointestinal tract (35,36) and the observation that  $\beta_3$  agonists inhibit in vitro colon contraction bioassays (37). On the other hand, a  $\beta_3$  agonist slows intestinal motility in mice lacking all  $\beta_3$  receptors except those in adipose tissue (23). This observation plus our present data suggest that stimulation of  $\beta_3$  receptors in adipose tissue is required for production of a signal that causes inhibition of gastrointestinal motility.

**Species differences in  $\beta_3$  physiology.** Differences in  $\beta_3$  physiology between humans and mice have been identified both in ligand binding specificity (8) and in WAT mRNA

expression levels (38). Even  $\beta_3$  knockout mice are only slightly fatter than controls (22). Given these differences, caution must be used when extrapolating  $\beta_3$  conclusions from mice to humans.

In conclusion, the inability of  $\beta_3$  stimulation to increase energy metabolism in the A-ZIP/F-1 mice is probably because of intrinsic and acquired BAT defects and because of insufficient WAT triglyceride stores. Our results demonstrate that lipoatrophic A-ZIP/F-1 mice are deficient in all measured responses to  $\beta_3$ -adrenergic agonist treatment, including insulin secretion and intestinal motility, which indicates that adipose tissues are required for all of these responses.

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