

Improved Glucose and Lipid Metabolism in Genetically Obese Mice Lacking aP2

K. TEOMAN UYSAL, LUDGER SCHEJA, SARAH M. WIESBROCK,
SUSAN BONNER-WEIR, AND GÖKHAN S. HOTAMISLIGIL

Division of Biological Sciences and Department of Nutrition (K.T.U., L.S., S.M.W., G.S.H.), Harvard School of Public Health, Boston, Massachusetts 02115; and Research Division (S.B.W.), Joslin Diabetes Center, Boston, Massachusetts 02215

ABSTRACT

Adipocyte fatty acid-binding protein, aP2, is a member of the intracellular fatty acid binding protein family. Previously, studies have shown increased insulin sensitivity in aP2-deficient mice with dietary obesity. Here, we asked whether aP2-related alterations in lipolytic response and insulin production are features of obesity-induced insulin resistance and investigated the effects of aP2-deficiency on glucose homeostasis and lipid metabolism in ob/ob mice, a model of extreme obesity. ob/ob mice homozygous for the aP2 null allele (ob/ob-aP2^{-/-}) became more obese than ob/ob mice as indicated by significantly increased body weight and fat pad size but unaltered body length. However, despite their extreme adiposity, ob/ob-aP2^{-/-} animals were more insulin-sensitive compared with ob/ob controls, as demonstrated by significantly lower plasma glucose and insulin levels

and better performance in both insulin and glucose tolerance tests. These animals also showed improvements in dyslipidemia and had lower plasma triglyceride and cholesterol levels. Lipolytic response to β -adrenergic stimulation and lipolysis-associated insulin secretion was significantly reduced in ob/ob-aP2^{-/-} mice. Interestingly, glucose-stimulated insulin secretion, while virtually abolished in ob/ob controls, was significantly improved in ob/ob-aP2^{-/-} animals. There were no apparent morphological differences in the structure or size of the pancreatic islets between genotypes. Taken together, the data indicate that in obesity, aP2-deficiency not only improves peripheral insulin resistance but also preserves pancreatic β cell function and has beneficial effects on lipid metabolism. (*Endocrinology* 141: 3388–3396, 2000)

OBESITY, with a prevalence around 55–63% (body mass index ≥ 25 kg/m²) in the United States population (1, 2), is a major risk factor for the development of type 2 diabetes and associated pathological states such as dyslipidemia, hypertension and atherosclerosis (3, 4). More than 80% of individuals with type 2 diabetes are obese (5). The pathogenesis of type 2 diabetes involves the progressive development of hyperinsulinemia and insulin resistance, a decreased response to insulin in target tissues (6). Another hallmark of the disease is impaired β -cell function that, at least in some cases, is already detectable in stages preceding overt hyperglycemia (7). Although the principal defects of type 2 diabetes are clear, how an expanded fat mass results in any of these pathologies is not well understood.

Possible factors for obesity-induced disorders are lipid molecules that are elevated in obesity, such as long chain nonesterified fatty acids (FFA; FFA). In agreement with a role of systemic FFA in the development of type 2 diabetes, it has been shown that elevation of plasma FFA induces peripheral insulin resistance in humans and rodent models within few hours (8–10). In addition, it has been shown that FFA can have positive or negative effects on insulin secretion, depending on the experimental conditions (11–18). Thus, obesity-induced elevation of systemic FFA might have direct impact on glucose homeostasis via systemic insulin sensitivity and possibly through effects on insulin secretion.

Adipocyte fatty acid-binding protein, aP2, is a member of the intracellular fatty acid binding protein family (19) highly expressed in adipocytes (20, 21). While the exact cellular functions of aP2 remain largely undefined, our earlier work has indicated an important *in vivo* role for this protein in lipid and glucose metabolism. Mice deficient for aP2 (aP2^{-/-}) were shown to be protected from development of insulin resistance and hyperinsulinemia in a high fat diet-induced obesity model, establishing aP2^{-/-} mice as an experimental system to study the pathogenesis of type 2 diabetes (22). In subsequent studies with lean animals, it was shown that the lipolytic response was attenuated in aP2^{-/-} mice and isolated aP2^{-/-} adipocytes (23, 24). Furthermore, lipolysis-associated insulin secretion in the lean aP2^{-/-} mice was profoundly reduced although the pancreatic response to other insulin secretagogues was unaltered (23). Although the mechanisms underlying these changes are not yet clear, the interesting possibility was raised that an intrinsically reduced propensity to secrete insulin in response to lipid or other mediators might contribute to the protection of aP2^{-/-} mice from hyperinsulinemia and consequently insulin resistance.

To test the relevance of these observations in obesity, in a uniform genetic setting, we generated ob/ob mice lacking aP2. In these animals, parameters relevant to obesity, insulin resistance, insulin secretion, and plasma lipids were studied longitudinally to assess the overall effect of aP2-deficiency on obesity-related metabolic abnormalities. Lipolysis, lipolysis-associated insulin secretion, and β cell function were studied in more detail in a subset of animals. Our results demonstrate that aP2-deficiency has significant beneficial effects on

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Address all correspondence and requests for reprints to: Gökhan S. Hotamisligil, M.D., Ph.D., Harvard School of Public Health, 665 Huntington Avenue, Boston, Massachusetts 02115. E-mail: ghotamis@hsph.harvard.edu.

insulin resistance in the ob/ob model, and that the lack of aP2 results in reduced lipolysis and lipolysis-induced insulin secretion in obesity.

Materials and Methods

Generation of ob/ob and ob/ob-aP2^{-/-} mice

Mice deficient in aP2 (backcrossed 12 times into C57BL/6 background) were intercrossed with animals heterozygote in the ob (leptin) locus (OB/ob C57BL/6) to generate double heterozygotes (OB/ob-aP2^{+/-}). These mice were then intercrossed to generate OB/ob-aP2^{-/-} or OB/ob-aP2^{+/+} mice, which subsequently acted as parents to lean and obese (OB/OB and ob/ob respectively) animals either wild-type (aP2^{+/+}) or null (aP2^{-/-}) in the aP2 locus. All mice that are homozygous for the ob mutation (ob/ob) developed obesity and hence, are referred to as obese in the text. Mice were kept on a 12-h light cycle, staying in dark between 1900 h and 0700 h and were fed either a standard mouse chow or a high-fat, high-carbohydrate diet *ad libitum* (Diet F3283, Bio-Serv, Frenchtown, NJ). Experiments and sample collection took place in the early afternoon after either a 6-hour daytime food withdrawal for steady state measurements or following a 24-h fast.

Metabolic measurements

Total body weights were measured monthly from age 4–16 weeks. Blood samples were collected at 4, 8, and 12 weeks of age. Glucose concentrations in plasma were measured using glucose analyzer glucose strips (Medisense, Bedford, MA). Serum insulin and C-peptide were measured with a monoclonal insulin RIA (Linco Research, Inc., St. Louis, MO). Glucose and insulin tolerance tests were performed on conscious male animals following a 24 h fast by ip administration of glucose (1.8 mg/g) and measurements of tail blood glucose at 15, 30, 45, 60, 90, and 120 min. The insulin tolerance test was done similarly except for the injection of recombinant mouse insulin (1 U/kg, Sigma, St. Louis, MO) and an additional blood glucose measurement at 150 min. Plasma tri-glycerides, glycerol, cholesterol, and FFA levels were measured using commercially available color enzymatic assays (Sigma and Wako Pure Chemical Industries Ltd., Richmond, VA). In both CL 316,243- (β 3-adrenoreceptor specific agonist provided by Dr. K. Steiner, Wyeth-Ayerst Laboratories, Inc., Princeton, NJ) stimulated lipolysis and glucose-stimulated insulin secretion experiments, the animals were fasted 24 h before the experiments. The compounds were dissolved in PBS and injected ip at a dose of 0.1 mg/kg (CL 316,243) and 1.8 mg/g (glucose). In all experiments, except the tolerance tests, blood was collected from the orbital plexus after anesthetizing animals with methoxyflurane (Mallinckrodt, Inc. Veterinary). Dynamic experiments were performed in males, whereas steady-state measurements were in both males and females. For all measurements, such as tolerance tests, CL 316,243 stimulated lipolysis, or glucose-stimulated insulin secretion, statistical analysis was performed using ANOVA repeated measurements.

Staining of pancreatic sections

Following administration of an overdose of sodium amytal, pancreas was excised from each animal *in toto*. After excision, each pancreas was lightly blotted, weighed, fixed in Bouin's fixative, and embedded in paraffin by routine techniques. Sections were immunostained (immunoperoxidase) to examine the β cells using antibodies against insulin (guinea pig anti-porcine insulin, Linco Research, Inc., St. Charles, MO, 1:200) or the non- β -endocrine cells of the islet (the mantle) using a cocktail of antibodies against glucagon (antibovine final dilution 1:3000, gift of Dr. M. Appel), somatostatin (rabbit antisynthetic final dilution 1:300, made in the laboratory of Dr. S. Bonner-Weir), and pancreatic polypeptide (rabbit antibody final dilution 1:3000, gift of Dr. R. Chance, Eli Lilly & Co., Indianapolis, IN). The sections were incubated overnight at 4 C, washed in PBS, incubated with goat antirabbit IgG as a secondary antibody, washed with Tris buffer (pH 7.4), incubated with a peroxidase antirabbit serum (Cappel Laboratories, Cochranville, PA), stained with 3,3'-diaminobenzidine tetrahydrochloride (Sigma Immunochemicals) and counterstained with hematoxylin. Insulin staining was done similarly except for the use of a goat anti guinea pig secondary

antibody. The slides were evaluated blindly to the genotype of the animals.

Results

Body weight and adiposity of mice

To assess the potential effects of aP2-deficiency on body weight regulation and energy metabolism, we monitored growth and total body weight of lean and obese aP2^{+/+} and aP2^{-/-} animals of both sexes for a period of 16 weeks. As shown in Fig. 1, no significant difference in body weight was observed between lean aP2-deficient and wild-type animals. However, throughout the study period, ob/ob mice deficient in aP2 (ob/ob-aP2^{-/-}) displayed increased obesity compared with obese control animals (ob/ob). At 16 weeks of age both male and female ob/ob-aP2^{-/-} mice weighed 15% more compared with ob/ob animals ($P < 0.001$).

To determine if the increased body weight in ob/ob-aP2^{-/-} mice was the result of increased axial growth, we measured the body length of these animals. No difference

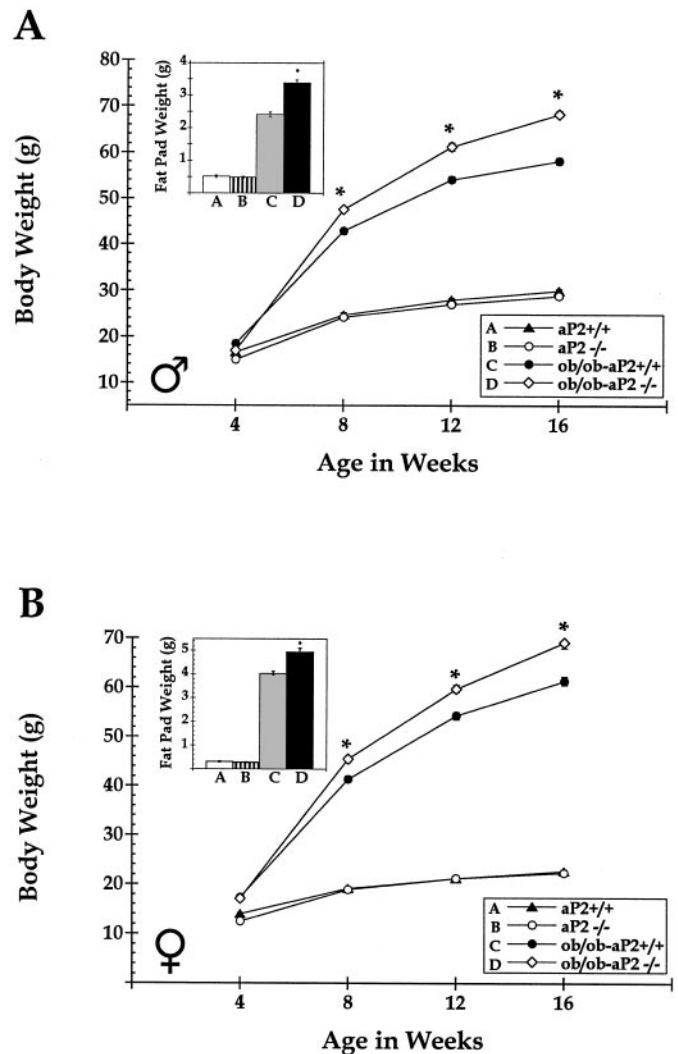


FIG. 1. Growth curves and fat pad weights of lean and obese aP2^{+/+} and aP2^{-/-} mice. Data from male (A) and female (B) mice are shown as mean \pm SE ($n = 27$ –36). * indicates $P < 0.05$ ob/ob-aP2^{-/-} vs. ob/ob-aP2^{+/+}.

was evident in the nasal-anal length of the aP2^{-/-} and aP2^{+/+} animals in either lean or obese group (data not shown). Epididymal or periovarian fat pad weight of each animal was also weighed to determine if a parallel increase is evident in adiposity (Fig. 1). No significant difference in fat pad weight was observed between lean aP2^{-/-} and aP2^{+/+} animals in both sexes. However, the fat pad weights of ob/ob-aP2^{-/-} mice of both sexes were significantly higher compared with ob/ob controls, suggesting increased adiposity in these animals (3.4 ± 0.08 vs. 2.4 ± 0.08 g in males and 4.9 ± 0.12 vs. 4.0 ± 0.08 g in females, *P* < 0.001).

Glucose homeostasis

To determine the effects of the absence of aP2 on glucose metabolism, we measured glucose and insulin levels in both steady (after 6-h day time food withdrawal) and fasted (24 h) states. The lean animals remained euglycemic throughout the study, and there was no significant difference in blood glucose between lean aP2^{-/-} and aP2^{+/+} mice in either condition (Table 1). Similarly, the plasma insulin levels of all lean mice were within normal boundaries in the fed state. In the fasted state, insulin levels of male aP2^{-/-} mice were higher than that of the aP2^{+/+} animals, but this difference did not reach statistical significance in the females. In all obese groups, the animals developed hyperglycemia and hyperinsulinemia compared with the lean controls. However, both conditions were significantly improved in the aP2^{-/-} animals and in both sexes, ob/ob-aP2^{-/-} displayed lower plasma glucose and insulin levels, indicating a better glucose homeostasis. Overall, plasma glucose levels in ob/ob-aP2^{-/-} mice were decreased by 20–25% in both sexes compared with ob/ob controls (*P* < 0.001). Similarly, a 35–40% reduction in plasma insulin levels was evident in ob/ob-aP2^{-/-} in both conditions and in both sexes.

To determine insulin sensitivity directly in lean and obese aP2^{-/-} mice and controls, ip insulin and glucose tolerance tests were performed (Fig. 2). The deficiency of aP2 had no effect on insulin sensitivity in the lean groups. However, in obese animals, the ob/ob-aP2^{-/-} mice displayed a signifi-

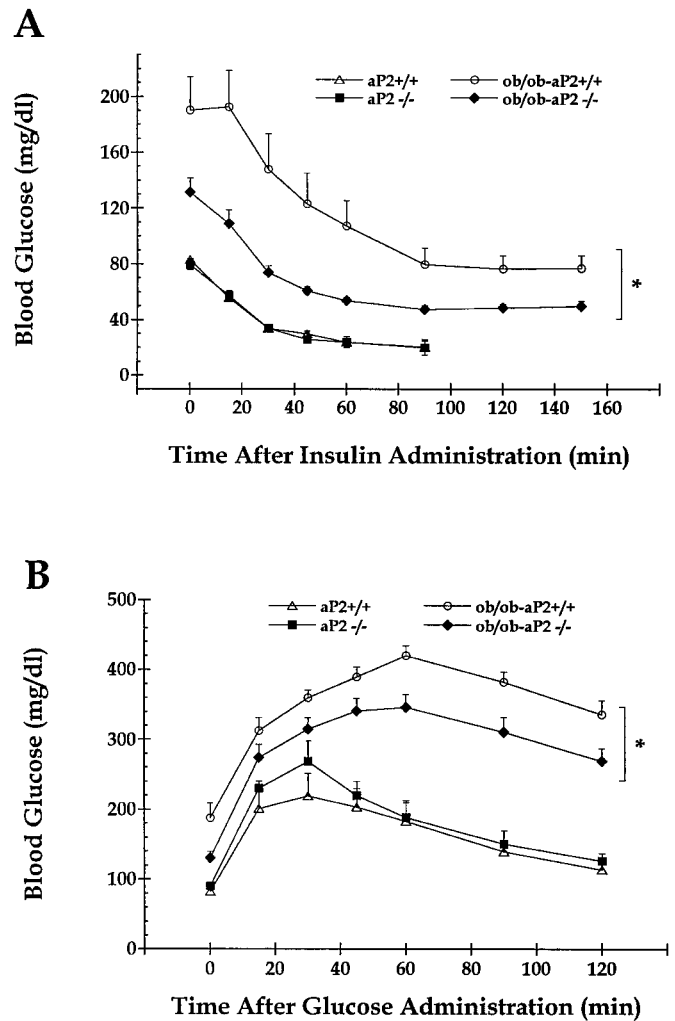


FIG. 2. Insulin (A) and glucose (B) tolerance tests in lean and obese aP2^{+/+} and aP2^{-/-} mice. *n* = 4, 4, 13, 19 for aP2^{+/+}, aP2^{-/-}, ob/ob-aP2^{+/+} and ob/ob-aP2^{-/-}, respectively. Data are shown as mean ± SE. * indicates *P* < 0.05 ob/ob-aP2^{-/-} vs. ob/ob-aP2^{+/+}.

TABLE 1. Plasma measurements of metabolic parameters in lean and obese aP2^{+/+} and aP2^{-/-} mice at 12 weeks of age

	aP2 ^{+/+}		aP2 ^{-/-}		ob/ob-aP2 ^{+/+}		ob/ob-aP2 ^{-/-}	
	Steady	Fasted	Steady	Fasted	Steady	Fasted	Steady	Fasted
Male								
Glucose (mg/dl)	155 ± 7	73 ± 2	149 ± 2	79 ± 3	290 ± 10	159 ± 11	237 ± 8 ^b	113 ± 4 ^b
Insulin (ng/ml)	1.8 ± 0.2	0.26 ± 0.03	2.1 ± 0.2	0.56 ± 0.14 ^a	10.5 ± 0.5	3.3 ± 0.2	6.7 ± 0.5 ^b	2.30 ± 0.1 ^b
FFA (mM)	0.45 ± 0.01	1.01 ± 0.03	0.50 ± 0.02	1.12 ± 0.02 ^a	0.48 ± 0.01	0.64 ± 0.04	0.55 ± 0.02 ^b	0.87 ± 0.02 ^b
Glycerol (mM)	0.20 ± 0.01	0.45 ± 0.01	0.18 ± 0.01 ^a	0.44 ± 0.01	0.28 ± 0.01	0.38 ± 0.03	0.23 ± 0.01 ^b	0.43 ± 0.01
Triglyceride (mg/dl)	46 ± 1	80 ± 2	44 ± 1	69 ± 3 ^a	63 ± 3	71 ± 5	48 ± 2 ^b	68 ± 3
Cholesterol (mg/dl)	88 ± 1	89 ± 2	91 ± 2	90 ± 2	183 ± 4	156 ± 6	175 ± 4	150 ± 8
Female								
Glucose (mg/dl)	140 ± 5	66 ± 1	134 ± 2	66 ± 3	252 ± 14	174 ± 16	209 ± 9 ^b	125 ± 5 ^b
Insulin (ng/ml)	2.0 ± 0.2	0.32 ± 0.10	2.2 ± 0.3	1.0 ± 0.2	11.1 ± 0.8	3.4 ± 0.9	6.9 ± 0.8 ^b	2.1 ± 0.3 ^b
FFA (mM)	0.50 ± 0.03	1.07 ± 0.05	0.50 ± 0.04	1.23 ± 0.04 ^a	0.51 ± 0.02	0.71 ± 0.07	0.62 ± 0.04 ^b	0.99 ± 0.06 ^b
Glycerol (mM)	0.23 ± 0.01	0.50 ± 0.02	0.19 ± 0.01 ^a	0.49 ± 0.01	0.28 ± 0.01	0.42 ± 0.01	0.23 ± 0.01 ^b	0.47 ± 0.03
Triglyceride (mg/dl)	40 ± 1	72 ± 6	37 ± 2	53 ± 1 ^a	47 ± 1	47 ± 2	41 ± 1 ^b	50 ± 1
Cholesterol (mg/dl)	85 ± 1	88 ± 2	83 ± 2	91 ± 3	173 ± 4	163 ± 6	162 ± 3 ^b	142 ± 2 ^b

In the steady-state, numbers were *n* = 27–36 for male and *n* = 27–31 for female animals. In the fasted state, numbers were *n* = 17–38 for male and *n* = 6–19 for female animals.

^a indicates *p* < 0.05 when aP2^{-/-} is compared with aP2^{+/+} mice.

^b indicates *p* < 0.05 when ob/ob-aP2^{-/-} is compared with ob/ob-aP2^{+/+} mice.

cantly stronger hypoglycemic response to a given dose of insulin compared with ob/ob controls, which is consistent with increased insulin sensitivity. Similarly, following the administration of glucose, ob/ob-aP2^{-/-} animals displayed improved glucose profiles consistent with increased insulin sensitivity compared with the ob/ob animals (Fig. 2).

Plasma cholesterol and triglyceride

The fact that increased plasma levels of triglyceride and cholesterol are important risk factors for atherosclerosis and are frequently associated with insulin resistance (3) prompted us to determine the plasma concentrations of triglycerides and cholesterol in lean and obese aP2^{-/-} and control mice. In the lean animals, we did not observe an effect of aP2-deficiency on plasma cholesterol levels (Table 1). However, female ob/ob-aP2^{-/-} mice had lower plasma cholesterol levels compared with ob/ob controls (10–15% decrease, $P < 0.01$). This difference was only 5% in males and was not statistically significant. aP2 deficiency also had moderate effects in reducing plasma triglyceride levels, under some but not all conditions. In the lean group, plasma triglyceride levels were decreased 15% in males and 26% in females ($P < 0.001$) in fasted animals but no significant difference was observed in the steady-state (Table 1). In contrast, ob/ob-aP2^{-/-} mice of both sexes had significantly lower steady state plasma triglyceride levels (25% in males and 10% in females, $P < 0.01$) compared with ob/ob controls.

Plasma glycerol and FFA

Increased FFAs are associated with obesity and have been postulated to be causally involved in the development of hyperinsulinemia and insulin resistance (10, 25, 26). To test if systemic FFA levels play a role in the observed improvement in insulin sensitivity in ob/ob-aP2^{-/-} mice, we measured plasma levels of FFA and glycerol, the two products of lipolysis, under both basal and CL 316,243-stimulated conditions. Baseline plasma glycerol levels were lower in aP2^{-/-} lean and obese animals of both sexes (Table 1). This decrease was 12–18% in the lean and 15% in the obese groups ($P < 0.04$). In the fasted state the glycerol levels increased in all mice and no difference was evident between aP2^{-/-} and aP2^{+/+} animals. In general, a small (10%, $P < 0.05$), but consistent increase was observed in the plasma FFA levels of aP2^{-/-} mice with the exception of lean animals in the steady-state (Table 1). In the obese group, the aP2-deficient mice also had higher plasma FFA levels (14–20% in the steady state and 26–29% after fasting, $P < 0.01$).

Lipolysis

Lipolysis is an adipocyte function that might contribute to the development of insulin resistance in obesity (27, 28). Thus, we examined *in vivo* lipolysis stimulated by CL-316,243 (0.1 mg/kg), a specific agonist of the β_3 -adrenergic receptor (β_3 -AR) (29), which is primarily expressed in adipose tissue and gastrointestinal tract (30, 31). For these experiments, blood samples were collected before and 8, 16, 30 and 60 min after the ip injection of 0.1 mg/kg CL 316,243. While plasma glycerol levels increased over basal levels in all animals, the

extent of this increase was significantly smaller in both lean and obese aP2-deficient mice (Fig. 3A). At 8 min, when the response is maximal, the rise in plasma glycerol levels was 2.2-fold in ob/ob-aP2^{-/-} mice compared with the 2.8-fold increase in ob/ob-aP2^{+/+} controls ($P < 0.05$). Similar to glycerol, FFA levels also increased in all animals following CL 316,243 administration reaching maximum levels at 30 min (Fig. 3B). This increase, however, was much smaller in both lean and obese aP2-deficient animals throughout the experiment. At 30 min, the plasma FFA levels of ob/ob-aP2^{-/-} animals increased by only 1.8-fold, whereas a 2.8-fold increase was evident in ob/ob controls indicating a reduced lipolytic response associated with aP2-deficiency.

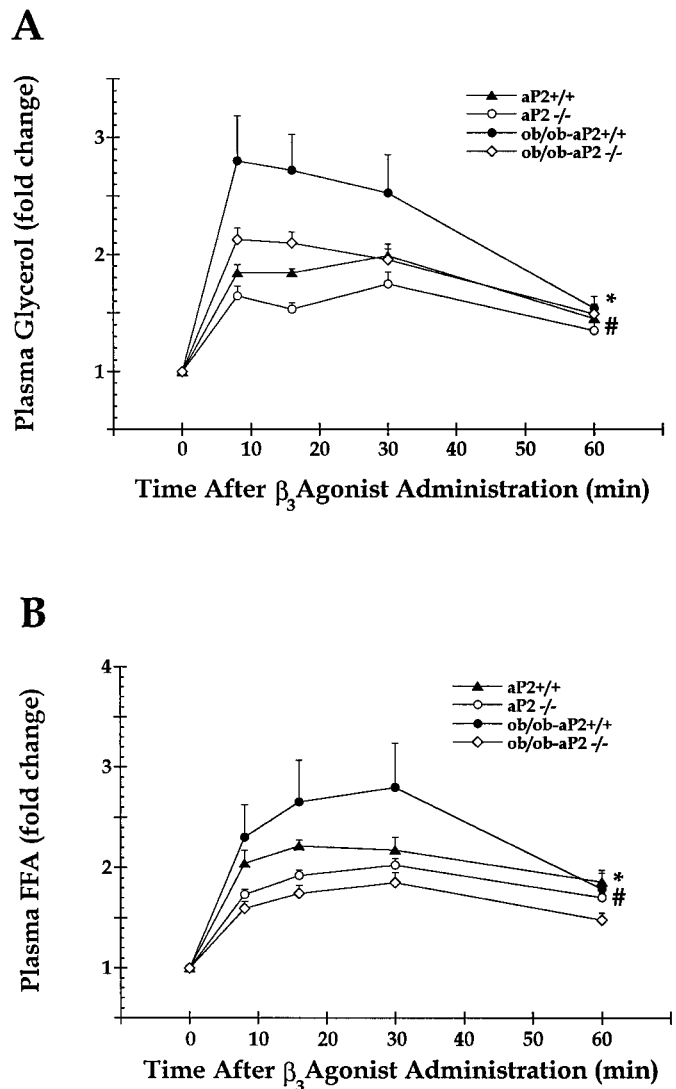


FIG. 3. Lipolysis in lean and obese aP2^{+/+} and aP2^{-/-} mice. Plasma glycerol (A) and FFA (B) were measured after administration of β_3 -adrenergic receptor agonist. Data are shown as mean \pm SE ($n = 10$ – 20). * indicates $P < 0.05$ ob/ob-aP2^{-/-} vs. ob/ob-aP2^{+/+}. # indicates $P < 0.05$ aP2^{-/-} vs. aP2^{+/+}. Basal plasma glycerol levels were 0.337 ± 0.02 , 0.40 ± 0.03 , 0.34 ± 0.04 and 0.42 ± 0.02 mM for aP2^{+/+}, aP2^{-/-}, ob/ob-aP2^{+/+} and ob/ob-aP2^{-/-}, respectively. Basal plasma FFA levels were 0.92 ± 0.05 , 1.03 ± 0.03 , 0.59 ± 0.05 , and 0.86 ± 0.03 mM for aP2^{+/+}, aP2^{-/-}, ob/ob-aP2^{+/+} and ob/ob-aP2^{-/-}, respectively.

Lipolysis-induced insulin secretion

Stimulation of adipocyte lipolysis *in vivo* is associated with increased insulin secretion (32, 33). Because alterations in this response could be related to the observed reduction of hyperinsulinemia in *ob/ob*- α P2^{-/-} animals, we studied β -AR-stimulated insulin secretion in lean and obese mice. For these experiments, blood samples were collected before and 8, 16, 30, and 60 min after ip administration of 0.1 mg/kg CL 316,243. Interestingly, in both lean and obese groups, we observed a dramatic reduction in the β 3-AR-stimulated insulin secretion in α P2^{-/-} mice compared with α P2^{+/+} controls (Fig. 4A). In the lean group, the plasma insulin levels started to increase by 8 min and reached a maximum at 30 min. At this time, the plasma insulin levels were increased by 24- and 52-fold in α P2^{-/-} and α P2^{+/+} mice, respectively. This difference between the lean groups subsided by 60 min. In the obese groups, a notable rise in circulating insulin levels was not detected until 30 min after stimulation. The increase in plasma insulin levels in the *ob/ob*- α P2^{-/-} (2- and 4-fold) was significantly smaller than *ob/ob* controls (4- and 6-fold) at 30 and 60 min, respectively. These data demonstrate that the insulin secretory response to lipolytic stimulation is strikingly reduced in α P2-deficient animals.

 β -cell function and morphology

Because we observed reduced β 3-AR-mediated insulin secretion in both lean and obese α P2^{-/-} mice, we asked whether this difference is the result of generally reduced β cell function or a specific unresponsiveness to only lipolytic stimuli. Hence, we examined the insulin secretion response to glucose in the obese and lean groups. In the lean animals, the magnitude of glucose-stimulated insulin and C-peptide secretion was not different between the α P2^{-/-} mice and α P2^{+/+} controls (Fig. 4, B and C). In the obese group, the overall response to glucose was not robust. Interestingly, α P2-deficiency rendered *ob/ob* mice more responsive to glucose, reflected in higher plasma insulin and C-peptide concentrations following glucose administration compared with control animals. One hour after glucose injection, plasma insulin levels of *ob/ob*- α P2^{-/-} mice was increased by 2.5-fold, whereas there was almost no change in plasma insulin levels of the *ob/ob* mice. Similarly, C-peptide levels increased 2-fold in *ob/ob*- α P2^{-/-} mice, whereas no response to glucose was evident in the *ob/ob* animals.

Finally, to determine whether any of the observed differences in insulin secretion were due to differences in pancreatic islet size or composition, we examined the pancreas of lean and obese α P2-deficient animals by immunohistochemistry. Islet morphology (size, shape, and organization of the non- β -cell mantle) was evaluated in blind fashion. Genotypes could not be separated on the basis of differences in pancreatic morphology. There was no significant difference in the pancreatic morphology or weight between wild-type and α P2-deficient mice (Fig. 5). No significant differences were also evident in the extent of islet degranulation in α P2^{-/-} and α P2^{+/+} obese animals compared with their lean counterparts. In addition, the obese animals, regardless of their genotype, had similar amounts of fat droplets scattered between the lobes and in the exocrine portion of pancreas (see Fig. 5).

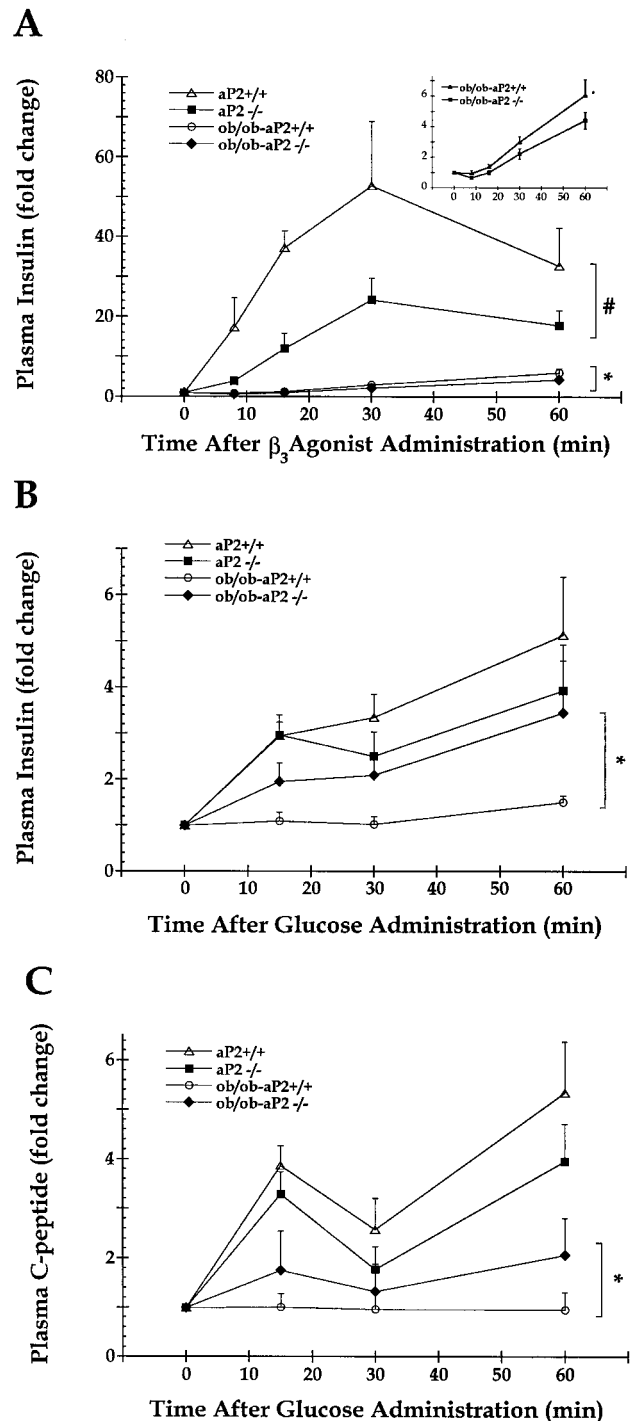


FIG. 4. Insulin secretion in lean and obese α P2^{+/+} and α P2^{-/-} mice. Plasma insulin levels were measured after administration of β 3-adrenergic receptor agonist (A) or glucose (B). We also measured plasma C-peptide levels after administration of glucose (C). Data are shown as mean \pm SE (n = 9–20). * indicates $P < 0.05$ *ob/ob*- α P2^{-/-} vs. *ob/ob*- α P2^{+/+}. # indicates $P < 0.05$ α P2^{-/-} vs. α P2^{+/+}. In (A), the basal plasma insulin levels were 0.14 ± 0.01 , 0.20 ± 0.01 , 3.70 ± 0.41 , and 2.41 ± 0.30 ng/ml for α P2^{+/+}, α P2^{-/-}, *ob/ob*- α P2^{+/+} and *ob/ob*- α P2^{-/-}, respectively. In (B), the basal plasma insulin levels were 0.39 ± 0.02 , 0.40 ± 0.02 , 2.2 ± 0.2 and 1.4 ± 0.1 ng/ml for α P2^{+/+}, α P2^{-/-}, *ob/ob*- α P2^{+/+} and *ob/ob*- α P2^{-/-}, respectively. Basal plasma C-peptide levels were 165 ± 20 , 228 ± 35 , 840 ± 68 and 816 ± 56 pm for α P2^{+/+}, α P2^{-/-}, *ob/ob*- α P2^{+/+}, and *ob/ob*- α P2^{-/-}, respectively.

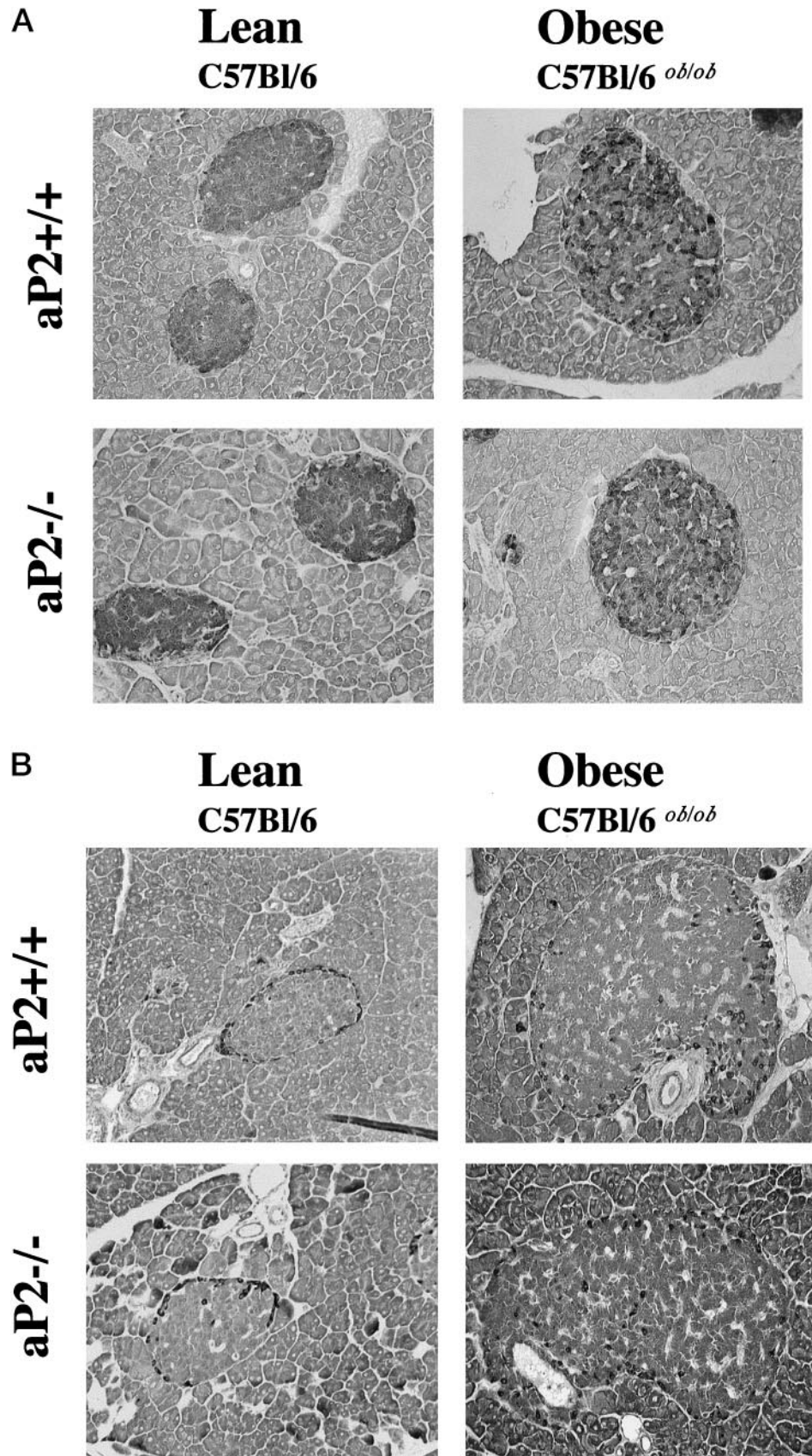


FIG. 5. Islet size and morphology in lean and obese $\alpha P2^{+/+}$ and $\alpha P2^{-/-}$ mice. Immunostaining of pancreatic sections were carried out using antibodies to insulin (A) and non- β -cell hormones (B). Representative sections are shown from all groups studied (n = 4–6).

Discussion

Obesity is ubiquitously associated with insulin resistance. More than 80% of the individuals with type 2 diabetes are also obese and an overwhelming majority of obese individuals express abnormalities of various degrees in insulin action and glucose metabolism. While the close relationship between obesity and diabetes has long been known, understanding the molecular mechanisms by which excess adiposity leads to this condition has been difficult.

Much attention has been placed on muscle tissue in systemic insulin resistance, both due to its mass and the fact that defective insulin action is detectable at this site early in the development of type 2 diabetes. Interestingly, recent studies with genetic rodent models have put unexpected weight on the role of adipose tissue and pancreatic islets as dominant sites impacting systemic insulin resistance and development of type 2 diabetes (34–39). In line with this notion are findings in mice genetically deficient in aP2, the adipocyte fatty acid-binding protein. The expression of aP2 is highly restricted to differentiated adipocytes (20, 21) and recently it has been detected in activated macrophages (Ref. 40 and our unpublished results). In a dietary model of obesity, aP2-deficiency leads to substantially increased insulin sensitivity (22), demonstrating that an isolated defect in the lipid biology of adipocytes might have a significant systemic effect on the course of obesity-induced hyperinsulinemia and insulin resistance.

How could an alteration in adipose lipid metabolism effect systemic glucose metabolism? A potential scenario is that expansion of fat tissue and concomitant insulin resistance of adipocytes will lead to increased lipolysis and release of byproducts such as FFA and cytokines from adipose tissue, eventually resulting in reduced glucose disposal and increased hepatic glucose production. This then stimulates the secretion of insulin to compensate for insulin resistance. Hyperinsulinemia further promotes insulin resistance at target sites through receptor desensitization and, indirectly, through its effects on lipogenesis. This vicious cycle will eventually result in chronic hyperglycemia, defective glucose-stimulated insulin secretion, β -cell pathologies and development of frank diabetes. According to this model, one potential strategy to disturb this vicious cycle will be to enhance insulin action in adipose tissue and prevent excess adipose tissue lipolysis. If the output of the adipose tissue is a critical and early stimulus for hyperinsulinemia in obesity, then decreased lipolysis and the associated products should be associated with lower rates of insulin secretion, and preservation of the β cell response to glucose.

Interestingly, in studies using lean animals, our group as well as Coe *et al.*, have recently demonstrated decreased lipolysis in aP2^{-/-} adipocytes *in vitro* and aP2^{-/-} mice *in vivo* (23, 24). The molecular mechanisms underlying impaired lipolysis in the absence of aP2 are not yet clear. In adipose tissue of aP2-deficient mice, there does not appear to be any quantitative defect in the major components of the lipolytic machinery. Interestingly, recent studies demonstrated that aP2 directly interacts with hormone sensitive lipase (HSL), raising the possibility that this interaction is critical in the efficiency of this enzyme (41).

During studies on lipolysis, we also made the intriguing observation that lipolysis-associated insulin secretion was dramatically reduced in lean aP2^{-/-} mice while the response to other insulin secretagogues remained intact (23). These observations prompted us to postulate that the decreased lipolysis and the subsequent insulin secretory response to the lipolytic products of the adipocyte might underlie the improved overall glucose homeostasis in obese aP2^{-/-} animals. To directly test this hypothesis, we have generated genetically obese mice by intercrossing aP2^{-/-} animals with the ob/ob mice, to establish a well-defined obesity model with aP2-deficiency.

In support of earlier observations in diet-induced obesity, this study clearly demonstrated that aP2-deficiency is associated with significantly improved glucose and lipid metabolism even in the presence of extreme obesity resulting from leptin deficiency. Although ob/ob-aP2^{-/-} mice were even heavier than the ob/ob-aP2^{+/+} animals, they displayed a much improved glucose metabolism compared with the obese controls. This was manifested in significantly lower plasma glucose and insulin levels throughout the experimental period and by their better performance in insulin and glucose tolerance tests. The insulin-sensitizing effect of aP2-deficiency was limited to the obese state, as we did not observe a trend toward higher insulin sensitivity in lean aP2^{-/-} animals. These improvements were, however, not complete since the ob/ob-aP2^{-/-} mice still displayed insulin resistance compared with lean animals.

Next, we tested whether aP2-deficiency leads to alterations in lipolysis and β cell responses in the presence of severe obesity. First, we demonstrated that the lipolytic response to β 3-adrenergic receptor stimulation is significantly decreased in lean and, even more so, in the obese aP2^{-/-} mice compared with control animals. This was shown by a reduction in the levels of both glycerol and FFA levels upon β 3-AR stimulation in aP2^{-/-} and ob/ob aP2^{-/-} mice. In our previous studies, we have observed significant alterations in glycerol but not in FFA levels in lean animals upon lipolytic stimuli. The extent of this response appears to be related to the metabolic state of the animals because, as shown here, 24 h fasting led to decreased responses in both glycerol and FFA, whereas our previous experiments, performed in the day time resting state, led only to decreased glycerol response (23).

Second, we demonstrate that in both lean and obese mice the lipolysis-associated increase in plasma insulin was substantially reduced in aP2^{-/-} animals compared with their respective controls following β 3-adrenergic stimulation. Similar results were also observed in aP2^{-/-} mice made obese by a high fat diet (data not shown). These alterations were not related to a pancreatic defect or general unresponsiveness of the islets. No significant alterations were evident in the morphology of the pancreatic β cells between aP2^{-/-} and aP2^{+/+} animals. The glucose-stimulated insulin secretion was normal and comparable to the lean mice. Most strikingly, glucose-stimulated insulin secretion was partially preserved in ob/ob aP2^{-/-} mice, whereas it was completely abolished in ob/ob controls. These data are consistent with the increased glucose tolerance in these animals and show that the beneficial effect of aP2 deficiency is not only

manifested in enhanced peripheral insulin sensitivity but also in better maintained pancreatic β cell function, both of which potentially contribute to improved glucose metabolism.

The mechanisms by which α P2-deficiency leads to improved metabolic control in obesity are not known. Our studies in the dietary obesity model have suggested that lack of obesity-induced TNF α production in adipose tissue might be part of this improved response (22). However, in the more severe obesity model, our preliminary studies did not demonstrate a significant reduction in TNF α expression in ob/ob- α P2 $^{-/-}$ animals suggesting that there are additional pathways modified by the absence of α P2 leading to improved insulin sensitivity (data not shown). Because α P2 binds to cytosolic fatty acids and could modulate their intracellular concentrations, availability or subcellular trafficking, we have also postulated that in the absence of this fatty acid binding activity, the activity of nuclear hormone receptors that are regulated by fatty acid ligands could be altered. Because the PPAR family of nuclear hormone receptors play a dramatic role in glucose and lipid metabolism, they will be the prime candidates for further examination. Although our preliminary analysis have not yet yielded support for this hypothesis, further studies will be necessary to definitively address this possibility.

In any case, data presented here further support the concept of an adipopancreatic axis, the function of which is specifically altered in the absence of adipocyte α P2. How α P2-deficiency leads to reduction in lipolysis-associated insulin secretion and provides some protection from deterioration of glucose-stimulated insulin secretion in the context of obesity remains to be shown. For the development of the latter pathological state, chronic elevation of systemic FFA has been proposed as a causal signal (14). In this study, we have seen a small but consistent increase in basal plasma FFA levels in both lean and obese α P2 $^{-/-}$ mice, which argues against a potential role for these molecules. On the other hand, lipolytic response is reduced, and the temporal and spatial dynamics of FFA output might still be altered and play a critical role for this physiological state. It is also possible that although small in magnitude, the elevation in basal FFA levels is sufficient to insensitize the islets to further lipid-derived stimuli. In any case, it is clear that the phenotype of the α P2 $^{-/-}$ mice makes these animals a suitable model to study the interaction between adipocytes and the islets and qualifies α P2 as a very interesting drug target for the treatment of insulin resistance, dyslipidemia, and diabetes.

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