Published in final edited form as:
Diabetologia. 2011 November ; 54(11): . doi:10.1007/s00125-011-2263-9.

Partial inactivation of *Ankrd26* causes diabetes with enhanced insulin responsiveness of adipose tissue in mice

G. A. Raciti[1], **T. K. Bera**[1], **O. Gavrilova**[2], and **I. Pastan**[1,3]

[1]Laboratory of Molecular Biology, Center for Cancer Research, National Cancer Institute, National Institutes of Health, Bethesda, Maryland, USA

[2]Mouse Metabolism Core, National Institute of Diabetes, Digestive and Kidney Diseases, National Institutes of Health, Bethesda, Maryland, USA

Abstract

Aims/hypothesis—*Ankyrin repeat domain 26* (*ANKRD26*) is a newly described gene located at 10p12 in humans, a locus identified with some forms of hereditary obesity. Previous studies showed that partial inactivation of *Ankrd26* causes hyperphagia, obesity and gigantism in mice. We hypothesized that *Ankrd26* mutant (MT) mice could develop diabetes and we sought to establish if the observed phenotype could be solely related to the development of obesity or could be caused by a direct action of Ankrd26 in peripheral tissues.

Methods—To test the hypothesis, a full metabolic characterization of the *Ankrd26* MT mice under *ad libutim* feeding or placed under two different calorie restricted dietary regimens was completed.

Results—Highly obese *Ankrd26* MT mice develop an unusual form of diabetes in which white adipose tissue (WAT) is insulin sensitive, while other tissues are insulin resistant. When obese MT mice were placed on a food-restricted diet their weight and glucose homeostasis returned to normal. Additionally, when young MT mice were placed on a pair-feeding diet with normal mice, they maintained a normal body weight but showed better glucose tolerance than normal mice, an increased responsiveness of WAT to insulin and enhanced phosphorylation of the insulin receptor.

Conclusions/interpratation—These findings show that the Ankrd26 protein has at least two functions in mice. One is to control the response of WAT to insulin and the other is to control appetite, which when mutated leads to hyperphagia and diabetes in an obesity-dependent manner.

Keywords

Ankrd26; calorie restriction; diabetes; insulin sensitivity; obesity; WAT

Introduction

Type 2 diabetes is the most common type of diabetes and accounts for 90% of all forms of diabetes. It is a heterogeneous syndrome that is due to the interaction of environmental factors with a genetic susceptibility to the disease [1], and is characterized by insulin

[3]Corresponding author: Laboratory of Molecular Biology, National Cancer Institute, 37 Convent Drive, Room 5106, Bethesda, MD 20892-4264 USA, Tel: (301) 496-4797; Fax: (301) 402-1344; pastani@mail.nih.gov.

Contribution statement G.A.R. designed the study, researched data, contributed to the discussion and wrote the manuscript; T.K.B. researched data and contributed to the discussion; O.G. researched data and contributed to the discussion; and I.P. designed the study and wrote the manuscript.

Duality of interest The authors declare that there is no duality of interest associated with this manuscript.

resistance and/or abnormal insulin secretion, either of which may predominate [2]. The prevalence of type 2 diabetes is rapidly increasing worldwide and is expected to affect approximately 366 million people by the year 2030 [1]. Obesity is a major risk factor for the development of type 2 diabetes. Indeed, type 2 diabetes is most common in people older than 45 who are overweight and, as a consequence of increased obesity among young people, it is becoming also more common in children and young adults [3]. Hereby "Diabesity" is a new term used to indicate diabetes occurring in the context of obesity [4] that describes one of the main threats to human health in the 21st century [5].

We recently described a new model of obesity due to partial inactivation of the *Ankyrin repeat domain 26* (*Ankrd26*) gene [6] that is located at 10p12 in humans, a genetic locus related to some forms of hereditary obesity [7]. The gene encodes a ~190 KDa protein highly expressed in the hypothalamus and other regions of the brain, as well as in many tissues and organs including liver, skeletal muscle and white adipose tissue (WAT) (unpublished data). The ANKRD26 protein is associated with the inner aspect of the cell membrane and contains both ankyrin repeats and spectrin helices, motifs known to interact with signaling proteins [6]. Mice with inactivation of the *Ankrd26* gene have marked hyperphagia, which results in extreme obesity and an increase in body length [6]. Furthermore, they also have high leptin levels suggesting a defect in the feeding centers in the brain.

In the present work, we sought to establish if these mutant (MT) mice develop diabetes and if the observed phenotype is solely related to the development of obesity or is caused by a direct action of Ankrd26 in peripheral tissues.

Methods

Mouse models and calorie restriction diets

Ankrd26 MT mice were backcrossed for 8 generations on the C57/Bl6 background in the animal facility of the NCI. Male *Ankrd26* homozygous MT mice and their normal littermates (wild type; WT) were used for all studies. All procedures were conducted in accordance with NIH guidelines, as approved by the Animal Care and Use Committees of the NCI and the NIDDK. Mice were housed one per cage on a 12-h light/dark cycle (lights on 0600–1800) and fed water and NIH-07 diet (11% calories from fat; Zeigler Brothers Inc., Gardners, PA) *ad libitum* (AL) when they were not under calorie restriction dietary regimens. For the food restricted (FR) diet, 4-month-old WT (n= 12) and *Ankrd26* MT (n= 12) mice were randomly assigned to two groups: AL and FR. We determined baseline daily food consumption by weighing the food provided and correcting for food not eaten, including spillage. FR was performed for 15 weeks and each FR mouse was provided with an allotment of food equal to 33% AL (baseline) daily consumption of MT mice (3 grams of food). For the pair feeding (PF) diet, 4-week-old WT (n= 12) and *Ankrd26* MT (n= 12) mice were randomly assigned to two groups: AL and PF. The PF was performed for a period of 6 months and each PF mouse was provided with an allotment of food equal to the amount of food eaten the day before by the control AL group. For both diets, food was supplied twice a day from Monday to Friday and once on Saturday and Sunday. Weight gain and body length for individual mice were measured as described [6]. Biochemical assays were measured as reported in ESM Methods.

Glucose and insulin tolerance, assessment of insulin and glucagon secretion, and hyperinsulinemic-euglyemic clamps

Glucose tolerance tests (GTTs), insulin tolerance tests (ITTs), and insulin secretion were measured as described [8]. Glucagon secretion was measured as described [9].

Hyperinsulinemic-euglycemic clamps were performed as previously described [10] and reported in the ESM Methods.

In vivo analysis of insulin signaling

Mice were fasted overnight, anesthetized, and injected i.p. with saline or insulin (10 U•kg body weight^{-1}). Ten min after injection, muscle, adipose and liver were removed and frozen in dry ice. Tissues homogenates and cell lysates were separated by SDS-PAGE and analyzed by western blot as previously described [8, 11, 12]. Membranes were probed with antibodies to phospho-IGF-I receptor beta (Tyr-1135/1136)/Insulin Receptor (IR) beta(Tyr-1150/1151), IR beta, phospho-Akt (Ser473), and Akt (Cell Signaling Technology, Danvers, MA).

Statistical analysis

Data are expressed as means ± SEM and statistical significance between groups was analyzed by 2-tailed Student's t test or analysis of variance (ANOVA) as appropriate. *P* values of <0.05 were considered statistically significant. The total AUC and the inverse AUC for glucose response during GTT and ITT were calculated using the following equations [13]:

$$\mathrm{AUC}=\sum_{n=1}^{x-1}\left((T_{n+1}-T_n)\times\left(\frac{\left|\mathrm{Glc}_{T_n}-\mathrm{Glc}_{T_{n+1}}\right|}{2}+\min\left(\mathrm{Glc}_{T_n},\mathrm{Glc}_{T_{n+1}}\right)-\mathrm{Glc}_{T_0}\right)\right);$$

$$\mathrm{AUC_i}=\sum_{n=1}^{x-1}\left((T_{n+1}-T_n)\times\left(\frac{\left|\mathrm{Glc}_{T_n}-\mathrm{Glc}_{T_{n+1}}\right|}{2}+\mathrm{Glc}_{T_0}-\max\left(\mathrm{Glc}_{T_n},\mathrm{Glc}_{T_{n+1}}\right)\right)\right)$$

Results

Ankrd26 mutant mice develop diabetes

To investigate the role of the *Ankrd26* gene in the regulation of glucose homeostasis *in vivo*, we did a full metabolic characterization of the *Ankrd26* MT mice. As reported previously [6], male *Ankrd26* MT mice were heavier and longer than age-matched controls (WT) at 2, 4 and 6 months (Table 1), and their daily food intake was 40% higher (WT: 3.5 ± 0.1 g/day; *Ankrd26* MT: 5.0 ± 0.1 g/day; $P < 0.001$). At 2 months of age *Ankrd26* MT mice exhibited a slight increase of both fasting and random fed blood glucose levels (Table 1), and showed a normal GTT (Fig. 1a). By contrast, both fasting and random fed blood glucose levels rose in the following months reaching a fasting level of 11.1 mmol/l at 6 months (Table 1). At 4 months of age glucose loading (2 g•kg body weight^{-1}) made the *Ankrd26* MT mice significantly more hyperglycemic during the following 120 min compared to control mice (Fig. 1c), and their glucose tolerance had severely deteriorated at 6 months (Fig. 1e), showing that partial inactivation of *Ankrd26* can lead to diabetes. Interestingly, the obese MT mice had elevated fasting insulin and leptin levels, yet there were no significant differences in fasting NEFA and triacylglycerol concentrations at 2, 4 and 6 months (Table 1).

Ankrd26 mutant mice show impaired insulin sensitivity and pancreatic functionality

To verify if *Ankrd26* inactivation is accompanied by reduced insulin sensitivity, we performed an ITT. Following i.p. injection of insulin (0.75 U•kg body weight^{-1}) there was a slight reduction of the hypoglycemic response in *Ankrd26* MT mice both at 2 and 4 months (Fig. 1b and d), and the hypoglycemic response was almost abolished in 6-month-old *Ankrd26* MT mice (Fig. 1f), showing a time-dependent deterioration of insulin sensitivity in the MT mice.

To assess pancreatic function, we evaluated both glucose-induced insulin secretion and insulin-induced glucagon secretion. In control mice, a 3-fold increase in insulin secretion was observed 3 min after glucose injection (3 g•kg body weight^{-1}), and the levels remained higher than baseline values for up to 30 min, indicating a second-phase response (Fig. 1g); furthermore, plasma glucagon levels were significantly increased by the hypoglycemic response induced by insulin (0.75 U•kg body weight^{-1}) (Fig. 1h). In contrast, both the acute first-phase insulin secretory response to glucose and the late second-phase response were completely abolished in *Ankrd26* MT mice (Fig. 1g). In addition, a defective glucagon response to hypoglycaemia was observed (Fig. 1h), indicating the concomitant presence of impaired insulin and glucagon secretion.

Hyperinsulinemic-euglycemic clamp in *Ankrd26* mutant mice

To analyze glucose metabolism and insulin sensitivity of *Ankrd26* MT mice in more detail, we performed a hyperinsulinemic-euglycemic clamp. In the basal state, *Ankrd26* MT mice had significantly increased plasma glucose (Fig. 2a). During the clamp, both the glucose infusion rates (GIRs) needed to maintain euglycaemia and whole-body glucose disposal rates (Rd) were significantly decreased in *Ankrd26* MT mice, indicating the presence of whole body insulin resistance (Fig. 2b–d). We also evaluated endogenous glucose production (EGP) and glucose uptake in the peripheral tissues. Insulin was not able to suppress EGP in *Ankrd26* MT mice (Fig. 2e), indicating hepatic insulin resistance. In addition, glucose uptake in skeletal muscle and in brown adipose tissue (BAT) was significantly decreased (Fig. 2f and g), but surprisingly there was no significant difference in glucose uptake of WAT glucose (Fig. 2h).

In vivo insulin signaling in *Ankrd26* mutant mice

To evaluate at the signaling level if the progression of the whole body insulin resistance is related to the age of the mice, we injected i.p. insulin (10 U•kg body weight^{-1}) into 2-and 6-month-old fasted *Ankrd26* MT and control mice and assessed the activation of insulin signaling pathway by western blots in liver, skeletal muscle and WAT. In 2-month-old MT mice the insulin-stimulated phosphorylation of both IRbeta on Tyr-1150/1151 and of Akt on the Ser473 were comparable to the phosphorylation of WT mice in liver and skeletal muscle and increased in WAT (Fig. 3a–f), indicating that their insulin signaling was generically intact. On the other hand, in 6-month-old *Ankrd26* MT mice the insulin-induced phosphorylation of both IRbeta and Akt was markedly reduced in liver and skeletal muscle (Fig. 3g, h, k and i). However in WAT the insulin response was still slightly increased compared to normal mice (Fig. 3j and l), confirming the observation obtained in the clamp studies that WAT remains insulin responsive.

Food restriction diet normalizes body weight and glucose tolerance in *Ankrd26* mutant mice

To determine if the insulin resistance and impairment of the insulin secretion, that lead to the onset of diabetes in the *Ankrd26* MT mice, were secondary to the development of obesity, we analyzed the glucose homeostasis of MT mice under two different calorie restricted feeding regimens, a FR diet and a PF diet.

First FR was performed for 15 weeks in 4-month-old obese and glucose intolerant *Ankrd26* MT and in age-matched lean WT mice fed daily with 3 grams of food, in order to observe whether the impairment of the glucose tolerance could be reversed by the dietary treatment. After 1 month, the *Ankrd26* MT mice with the FR diet showed a 15% reduction of body weight and a 60% reduction of fasting leptin levels compared to starting values (ESM Table 1, ESM Fig. 1a). This decrease in body weight was accompanied by a significant decrease of both fasting and random-fed blood glucose levels, as well as of fasting insulin and NEFA

levels in *Ankrd26* MT mice, and these values were comparable to those of WT mice (ESM Table 1). Glucose levels during GTT performed after 2 months of diet were significantly lower in *Ankrd26* MT mice compared to their levels before FR, and their glycaemia was even lower than controls, indicating higher glucose disposal (ESM Fig. 1b and c). In addition, FR *Ankrd26* MT mice had a near normal response to insulin with values comparable to those of WT mice (ESM Fig. 1d and e), and their insulin response to glucose injection became the same as the normal mice (ESM Fig. 1f).

Pair feeding diet maintains normal body weight and improves glucose tolerance in *Ankrd26* mutant mice

To determine if an impairment of the glucose tolerance could occur in lean *Ankrd26* MT mice, PF was performed for 6 months in 4-week-old *Ankrd26* MT and WT mice, fed daily with the amount of food eaten the day before by the control group. PF *Ankrd26* MT mice were slightly heavier than PF age-matched control mice at 2 and 4 months, but no significant differences in body weight and fasting leptin were observed at 6 months (Table 2, Fig. 4a). Interestingly, these MT mice with a normal body weight had the same length as the WT mice indicating that the change in length is dependent on some factor present only in the obese MT mice (Table 2; ESM Fig. 2). At the age of 2, 4 and 6 months, PF *Ankrd26* MT mice had fasting and random fed blood glucose levels, as well as fasting insulin levels within the normal range of the control mice (Table 2); they did have a slight decrease in both NEFA and triacylglycerol levels compared to WT mice (Table 2). Glucose levels during GTT performed in 2-month-old mice were significantly decreased in PF *Ankrd26* MT mice compared to those of WT mice, and this decrease was also present in 4- and 6-month-old MT mice, indicating the presence of an improved glucose tolerance (Fig. 4 b–d). Injection of insulin evoked a comparable reduced hypoglycemic responses in both PF *Ankrd26* MT and WT mice at 2, 4 and 6 months (Fig. 4e–g); in addition, the glucose induced insulin secretion profile was comparable between PF MT and control mice (Fig. 4h). All these data from both the calorie restricted feeding regimens demonstrate that the severe impairment of glucose tolerance induced by partial inactivation of the *Ankrd26* gene is secondary to obesity. Thus, in calorie-restricted mice the inactivation of this gene does not impair glucose homeostasis but, by contrast, seems to improve it.

Improved WAT insulin sensitivity in calorie restricted *Ankrd26* mutant mice

We performed hyperinsulinemic-euglycemic clamps in *Ankrd26* MT mice under the two different caloric regiments. During the clamp both the FR and PF *Ankrd26* MT mice showed glucose levels, GIRs, Rd and insulin suppression of the EGP comparable to the values of their respective controls (ESM Fig. 3a–e, Fig. 5a–e). In addition, the insulin-stimulated glucose uptake in skeletal muscle and in the BAT of the calorie restricted MT mice was similar to controls (ESM Fig. 3f and g, Fig. 5f and g), but interestingly was increased in the WAT (ESM Fig. 3h, Fig. 5h).

Improved WAT insulin signaling in calorie restricted *Ankrd26* mutant mice

Consistent with these data was our finding that phosphorylated IRbeta and Akt levels in response to insulin were comparable to those of WT mice in the liver and skeletal muscle from PF *Ankrd26* MT mice and they were higher than those of WT mice in the WAT from these mice (Fig. 6a–f). Similar data were obtained in FR *Ankrd26* MT mice (ESM Fig. 4a–e). Altogether these data indentified WAT of the MT mice as the site responsible for the improvement of the glucose tolerance in the lean *Ankrd26* MT mice, indicating a role for this gene in the regulation of insulin sensitivity in adipocytes as well as controlling appetite in the brain.

Discussion

In the present work, we report that MT mice with a partial inactivation of *Ankrd26* gene, exhibit elevated fasting and random fed blood glucose levels and develop diabetes when they are severely obese. MT mice are also markedly hyperinsulinemic in the basal state, show a poor insulin response to a glucose challenge and a defective glucagon response to hypoglycaemia, and are severely insulin resistant upon ITT. These findings show that both impaired insulin action and secretion contribute to the abnormal glucose tolerance produced by partial inactivation of *Ankrd26* gene. Further, hyperinsulinemic-euglycaemic clamps and studies of insulin signaling in obese *Ankrd26* MT mice demonstrate the onset of an unusual form of whole body insulin resistance, in which WAT remains insulin sensitive, while the other insulin target tissues become insulin resistant. These results suggest that *Ankrd26* gene has a role in the control of insulin sensitivity in WAT.

Although the pathogenesis of type 2 diabetes is known to be complex, the process involves apart from the heightened genetic susceptibility of certain ethnic groups, environmental and behavioral factors such as a sedentary lifestyle, nutrition and obesity [14]. To establish the role of obesity in the glycaemic disorders observed in the *Ankrd26* MT mice, we made use of two different calorie restriction feeding strategies. In one approach mature obese MT mice were placed on a FR diet, since chronic moderate reduction in calorie intake (~20–40%) results in weight loss and improves whole-body glucose homeostasis in humans, rats, and mice by increasing peripheral insulin sensitivity and decreasing glycaemia, insulinemia and leptinemia [15–18]. We found that FR completely restores normal body weight in MT mice and improves glucose metabolism by normalizing whole body insulin sensitivity and preserving insulin secretion. Further, FR MT mice have increased insulin sensitivity in WAT.

In the other study young MT mice were placed on a PF diet to prevent the onset of obesity maintaining the body weight in a normal range [19]. We found that PF is sufficient to maintain normal body weight in MT mice; furthermore PF MT mice show improved glucose tolerance, normal insulin secretion and whole body insulin sensitivity and, like the FR mice, they show increased glucose uptake in WAT. It therefore seems that the onset of extreme obesity, due to the marked hyperphagia of *Ankrd26* MT mice, is essential for the derangement of glucose homeostasis and for the development of diabetes, since impairment of glucose homeostasis is reversed by dietary regimen and does not occur in MT mice when their food intake and body weight are the same as control mice. Thus, *Ankrd26* MT mice should be regarded as a good mouse model of obesity-induced diabetes. But in marked contrast to other obesity and diabetes models, such as leptin-deficient *ob/ob* mice, leptin receptor deficient *db/db* mice and the yellow agouti (A^y) MT mice [20], *Ankrd26* MT mice have whole body insulin resistance in the presence of normal insulin sensitivity in WAT, indicating that peripheral insulin resistance in liver, skeletal muscle and BAT together with a dysfunction of beta cell function is sufficient to impair the glucose homeostasis in obese mice. Additionally, *Ankrd26* MT mice under calorie restriction regimens show a better glucose tolerance than control mice that is probably due to increased insulin sensitivity of WAT. Indeed, during clamp experiments insulin stimulated glucose uptake was slightly higher in WAT of calorie restricted MT mice. Enhancement of glucose tolerance associated with increased glucose disposal in WAT has been described in several adipose tissue specific transgenic mice [21–23]. For example, overexpression of the insulin responsive glucose transporter (GLUT4) in WAT causes enhanced glucose tolerance and increased glucose uptake in adipose tissue and leads to an increase in fat cell number [21]. Further in these transgenic mice as with our mice, despite these marked effects at the adipose cell level, obesity induced by high fat feeding leads to decrease in glucose tolerance due to insulin resistance in skeletal muscle and liver, where the GLUT4 transgene is not expressed [22].

An additional example is overexpression of liver glucokinase in WAT where the increase of glucose phosphorylation in adipocytes leads to enhance glucose uptake and metabolism in WAT improving glucose tolerance *in vivo* [23]. Therefore, by itself an increase of glucose disposal in WAT could be sufficient to improve glucose tolerance in calorie restricted MT mice.

In addition to improve glucose tolerance, *Ankrd26* MT mice under both calorie restriction regimens show decreased levels of NEFA and triacylglycerol, and also these lipid concentrations are in the normal range in the obese *Ankrd26* MT mice. WAT plays a crucial role in buffering the flux of fatty acids in the bloodstream and insulin modulates this process suppressing the release of NEFA into the circulation and increasing triacylglycerol storage in WAT [24], and increase of NEFA release by WAT and subsequent lipid abnormalities in type 2 diabetes are strongly associated with insulin resistance [24, 25]. Thus, the observed decrease in NEFA and triacylglycerol concentrations in calorie restricted Ankrd26 MT mice could be explained by their increased insulin sensitivity in WAT.

Furthermore, an improvement in insulin signaling was observed in WAT from these mice. Indeed, an evaluation of insulin signaling cascade in WAT from both FR and PF *Ankrd26* MT mice showed an increased tyrosine phosphorylation of the beta subunit of IR [26] and an increased phosphorylation of Akt, a serine/threonine kinase involved downstream of the insulin signaling [27]. We recently demonstrated that mouse embryonic fibroblast (MEF) cells from *Ankrd26* MT mice have a higher rate of spontaneous adipogenesis than WT MEFs and their adipogenesis is greatly increased when exposed to a mixture of inducers [28]. These findings indicate a prominent role of the *Ankrd26* gene in fat cells, regulating both their differentiation and insulin sensitivity. Unfortunately, we do not yet know how Ankrd26 can modulate insulin signaling and/or adipogenesis in fat cells. To detect proteins that interact with Ankrd26 points we are now doing a yeast two hybrid screen.

In addition to obesity, *Ankrd26* MT mice showed a 10% increase in linear growth even though circulating growth hormone (GH) and IGF1 are in the normal range [6]. Differences in body length were not detected in 4-week-old MT mice (ESM Fig. 2a), but were detected at 2 months of age in parallel with an increase in both body weight and serum leptin levels. It is well established that longitudinal growth is regulated by GH and its tissue mediator IGFI [29]. However recent evidence indicates that signals regulating energy balance also regulate the somatotrophic axis. For example leptin can promote longitudinal growth, since an impairment of leptin signaling causes reduced linear growth in both *ob/ob* and *db/db* mice [30], and leptin administration is a potent stimulator of bone growth in *ob/ob* mice [31]. In support of the hypothesis that leptin can play a crucial role in the increase of longitudinal growth in *Ankrd26* MT mice is our finding that PF MT mice with normal body weight and leptin levels exhibit longitudinal growth comparable to controls at 2, 4 and 6 months of age (ESM Fig. 2a and b).

Defects in *Ankrd26* gene expression or function play a critical role in controlling obesity in *Ankrd26* MT mice, since its partial inactivation causes hyperphagia and extreme obesity and does not cause a reduction in energy expenditure or activity [6]. In support of the important role of hyperphagia is our finding that MT mice with a post-weaning limitation of food intake have a normal body composition (fat and lean mass) (Table 2). The Ankrd26 protein is highly expressed in several regions of the brain, including the arcuate, paraventricular and ventromedial nuclei of the hypothalamus [6], a region known to play a key role in regulation of feeding behavior [32]. Moreover, obese *Ankrd26* MT mice do not show decreased food intake and body weight in response to leptin (3 mg•kg body weight^{-1}; data not shown), indicating the presence of a central leptin resistance. Therefore the obese phenotype of *Ankrd26* MT mice is very likely due to a prominent role of this protein in the regulation of

food intake in the brain. In support of this conclusion are the microarray analysis results obtained by Ko *et al.* who found that *Ankrd26* mRNA levels are increased in the hypothalamus of C57BL/6 mice after administration of three anti-obesity drugs (sibutramine, phendimetrazine, or methamphetamine), known to suppress appetite by activating catecholaminergic neurotransmission [33–36]. These findings lead the authors to suggest that the *Ankrd26* gene very likely accounts for the biological effects of these drugs. However, the precise mechanism by which *Ankrd26* controls food intake in MT mice is still not clear and is currently under investigation in our laboratory.

In summary, our results identify *Ankrd26* MT mice as a new model of obesity-induced diabetes since the inactivation of this gene *in vivo* can lead to diabetes, by impairing both insulin action and secretion, in an obesity-dependent manner. Furthermore, our results identify *Ankrd26* as a novel gene involved not only in the regulation of food intake, but also in the regulation of insulin responses in WAT. We propose Ankrd26 protein as an attractive molecular target for the generation of both new anti-obesity and new insulin sensitizing drugs.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

We thank William Jou and Tatyana Chanturiya (Mouse Metabolism Core, NIDDK) for invaluable technical support for the hyperinsulinemic-euglyemic clamp procedures. This research was supported in part by the Intramural Research Program of the NIH, NCI, Center for Cancer Research and in part by the NIDDK.

Abbreviations

AL	Ad Libitum
ANKRD26	Ankyrin Repeat Domain 26
BAT	Brown Adipose Tissue
DIPA	Delta Interacting Protein A
EGP	Endogenous Glucose Production
FR	Food Restriction
GH	Growth Hormone
GIR	Glucose Infusion Rate
GPS2	G-protein Pathway Suppressor 2
GTT	Glucose Tolerance Test
IR	Insulin Receptor
ITT	Insulin Tolerance Test
LAR	Protein Tyrosine Phosphatase
MEF	Mouse Embryonic Fibroblast
MT	Mutant
NCI	National Cancer Institute
NIDDK	National Institute of Diabetes, Digestive and Kidney Diseases

NIH	National Institutes of Health
PF	Pair Feeding
Rd	Whole-body Glucose Disposal Rate
RHO	Ras Homolog Gene Family
TRIO	Triple Functional Domain
WAT	White Adipose Tissue
WT	Wild Type

References

1. Gan, D., editor. Diabetes Atlas 2006. 3. International Diabetes Federation; Brussels: 2006.
2. Zimmet P, Alberti KG, Shaw J. Global and societal implications of the diabetes epidemic. Nature. 2001; 414:782–787. [PubMed: 11742409]
3. Fagot-Campagna A, Narayan K. Type 2 diabetes in children. Br Med J. 2001; 322:377–387. [PubMed: 11179142]
4. Astrup A, Finer N. Redefining type 2 diabetes: ‘diabesity’ or ‘obesity dependent diabetes mellitus’? Obes Rev. 2000; 1:57–59. [PubMed: 12119987]
5. Farag YM, Gaballa MR. Diabesity: an overview of a rising epidemic. Nephrol Dial Transplant. 2011; 26:28–35. [PubMed: 21045078]
6. Bera TK, Liu XF, Yamada M, et al. A model for obesity and gigantism due to disruption of the Ankrd26 gene. Proc Natl Acad Sci U S A. 2008; 105:270–275. [PubMed: 18162531]
7. Dong C, Li WD, Geller F, et al. Possible genomic imprinting of three human obesity-related genetic loci. Am J Hum Genet. 2005; 76:427–437. [PubMed: 15647995]
8. Miele C, Raciti GA, Cassese A, et al. PED/PEA-15 regulates glucose-induced insulin secretion by restraining potassium channel expression in pancreatic beta-cells. Diabetes. 2007; 56:622–633. [PubMed: 17327429]
9. Kawamori D, Kurpad AJ, Hu J, et al. Insulin signaling in alpha cells modulates glucagon secretion in vivo. Cell Metab. 2009; 9:350–361. [PubMed: 19356716]
10. Ozcan U, Yilmaz E, Ozcan L, et al. Chemical chaperones reduce ER stress and restore glucose homeostasis in a mouse model of type 2 diabetes. Science. 2006; 313:1137–1140. [PubMed: 16931765]
11. Guo T, Jou W, Chanturiya T, Portas J, Gavrilova O, McPherron AC. Myostatin inhibition in muscle, but not adipose tissue, decreases fat mass and improves insulin sensitivity. PLoS One. 2009; 4:e4937. [PubMed: 19295913]
12. Laemmli UK. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. Nature. 1970; 227:680–685. [PubMed: 5432063]
13. Heikkinen S, Argmann CA, Champy MF, Auwerx J. Evaluation of glucose homeostasis. Curr Protoc Mol Biol. 2007; 29(Unit 29B.3)
14. Zimmet P. Kelly West Lecture 1991. Challenges in diabetes epidemiology—from West to the rest. Diabetes Care. 1992; 15:232–252. [PubMed: 1547680]
15. Friedman JE, Dohm GL, Leggett-Frazier N, et al. Restoration of insulin responsiveness in skeletal muscle of morbidly obese patients after weight loss. Effect on muscle glucose transport and glucose transporter GLUT4. J Clin Invest. 1992; 89:701–705. [PubMed: 1737857]
16. Kelley DE, Wing R, Buonocore C, Sturis J, Polonsky K, Fitzsimmons M. Relative effects of calorie restriction and weight loss in noninsulin-dependent diabetes mellitus. J Clin Endocrinol Metab. 1993; 77:1287–1293. [PubMed: 8077323]
17. Dean DJ, Gazdag AC, Wetter TJ, Cartee GD. Comparison of the effects of 20 days and 15 months of calorie restriction on male Fischer 344 rats. Aging. 1998; 10:303–307. [PubMed: 9825021]

18. Harris SB, Gunion MW, Rosenthal MJ, Walford RL. Serum glucose, glucose tolerance, corticosterone and free fatty acids during aging in energy restricted mice. Mech Ageing Dev. 1994; 73:209–221. [PubMed: 8057691]
19. Cleary MP, Vasselli JR, Greenwood MR. Development of obesity in Zucker obese (fafa) rat in absence of hyperphagia. Am J Physiol. 1980; 238:E284–292. [PubMed: 7369356]
20. Carrolla L, Voisey J, van Daal A. Mouse models of obesity. Clin Dermatol. 2004; 22:345–349. [PubMed: 15475237]
21. Shepherd PR, Gnudi L, Tozzo E, Yang H, Leach F, Kahn BB. Adipose cell hyperplasia and enhanced glucose disposal in transgenic mice overexpressing GLUT4 selectively in adipose tissue. J Biol Chem. 1993; 268:22243–22246. [PubMed: 8226728]
22. Gnudi L, Tozzo E, Shepherd PR, Bliss JL, Kahn BB. High level overexpression of glucose transporter-4 driven by an adipose-specific promoter is maintained in transgenic mice on a high fat diet, but does not prevent impaired glucose tolerance. Endocrinology. 1995; 136:995–1002. [PubMed: 7867610]
23. Muñoz S, Franckhauser S, Elias I, et al. Chronically increased glucose uptake by adipose tissue leads to lactate production and improved insulin sensitivity rather than obesity in the mouse. Diabetologia. 2010; 53:2417–2430. [PubMed: 20623219]
24. Howard B. Lipoprotein metabolism in diabetes mellitus. J Lipid Res. 1987; 28:613–628. [PubMed: 3302085]
25. Zoratti R, Godsland I, Chaturvedi N, Crook D, Stevenson J, McKeigue P. Relation of plasma lipids to insulin resistance, non-esterified fatty acid levels and body fat in men from three ethnic groups: relevance to variation in risk of diabetes and coronary disease. Metabolism. 2000; 49:245–252. [PubMed: 10690953]
26. Patti ME, Kahn CR. The insulin receptor--a critical link in glucose homeostasis and insulin action. J Basic Clin Physiol Pharmacol. 1998; 9:89–109. [PubMed: 10212828]
27. Farese RV, Sajan MP, Standaert ML. Insulin-sensitive protein kinases (atypical protein kinase C and protein kinase B/Akt): actions and defects in obesity and type II diabetes. Exp Biol Med. 2005; 230:593–605.
28. Fei Z, Bera TK, Liu X, Xiang L, Pastan I. ANKRD26 gene disruption enhances adipogenesis of mouse embryonic fibroblasts. J Biol Chem. 2011 Epub ahead of print.
29. Le Roith D, Bondy C, Yakar S, Liu JL, Butler A. The somatomedin hypothesis: 2001. Endocr Rev. 2001; 22:53–74. [PubMed: 11159816]
30. Erickson JC, Hollopeter G, Palmiter RD. Attenuation of the obesity syndrome of ob/ob mice by the loss of neuropeptide Y. Science. 1996; 274:1704–1707. [PubMed: 8939859]
31. Steppan CM, Crawford DT, Chidsey-Frink KL, Ke H, Swick AG. Leptin is a potent stimulator of bone growth in ob/ob mice. Regul Pept. 2000; 92:73–78. [PubMed: 11024568]
32. King BM. The rise, fall, and resurrection of the ventromedial hypothalamus in the regulation of feeding behavior and body weight. Physiol Behav. 2006; 87:221–244. [PubMed: 16412483]
33. Ko MJ, Choi HS, Ahn JI, Kim SY, Jeong HS, Chung HJ. Gene expression profiling in C57BL/6 mice treated with the anorectic drugs Sibutramine and Phendimetrazine and their mechanistic implications. Genomics & Informatics. 2008; 6:117–125.
34. Chen TY, Duh SL, Huang CC, Lin TB, Kuo DY. Evidence for the involvement of dopamine D(1) and D(2) receptors in mediating the decrease of food intake during repeated treatment with amphetamine. J Biomed Sci. 2001; 8:462–466. [PubMed: 11702009]
35. Seiden LS, Sabol KE, Ricaurte GA. Amphetamine: effects on catecholamine systems and behavior. Annu Rev Pharmacol Toxicol. 1993; 33:639–677. [PubMed: 8494354]
36. Stock MJ. Sibutramine: a review of the pharmacology of a novel anti-obesity agent. Int J Obes Relat Metab Disord. 1997; 21(Suppl 1):S25–29. [PubMed: 9130038]

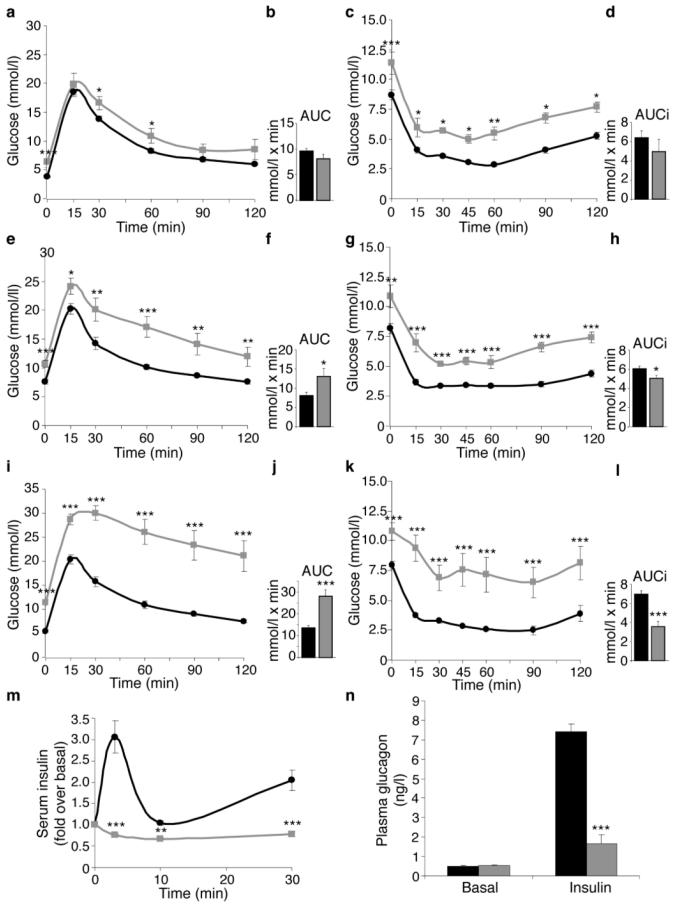

Fig. 1.

Glucose tolerance, insulin sensitivity, and insulin and glucagon secretion in *Ankrd26* mutant mice. Two- (**a**), 4- (**c**) and 6- (**e**) month-old *Ankrd26* MT (grey squares) and WT (black circle) mice were subjected to GTT. Mice were fasted for 16 hr and subjected to i.p. glucose loading (2 g•kg body weight^{-1}). Blood glucose levels were determined at various times. Mean AUC of MT (grey column) and WT (black column). Two- (**b**), 4- (**d**) and 6- (**f**) month-old MT (grey squares) and WT (black circle) mice were subjected to ITT. Random fed mice were injected i.p. with insulin (0.75 U•kg body weight^{-1}), followed by determinations of blood glucose levels at the indicated times. Mean AUCi of MT (grey column) and WT (black column). **g:** Glucose induced insulin secretion was evaluated in 6-month-old MT (grey squares) and WT (black circle) mice. Mice were fasted overnight and then injected i.p. with glucose (3 g•kg body weight^{-1}). Serum insulin concentrations were measured at the indicated times by ELISA. For each experiment (**a**–**g**), values are expressed as means ± SEM of determinations in at least 10 mice per group. **h)** Glucagon secretion was evaluated in 6-month-old random fed MT (grey column) and WT (black circle) mice. Mice were injected i.p. with insulin (0.75 U•kg body weight^{-1}). Plasma glucagon concentrations were measured before and 30 min after the insulin injection by RIA. Bars represent the means ± SEM of determinations in 5 MT and 6 control mice. *$P < 0.05$, **$P < 0.01$, and ***$P < 0.001$ vs. WT.

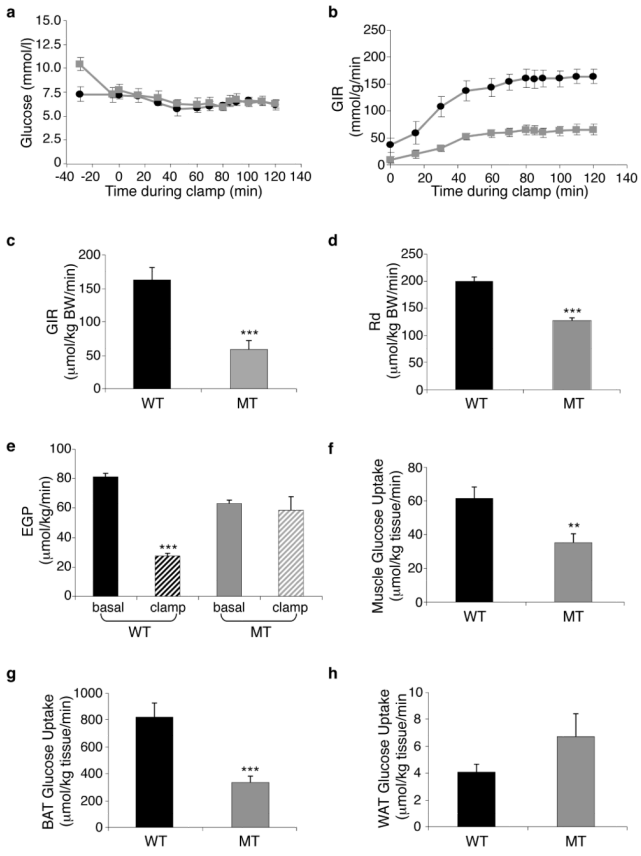

Fig. 2.

Hyperinsulinemic-euglycemic clamp studies in *Ankrd26* mutant mice. Hyperinsulinemic-euglycemic clamp studies were performed in 7-month-old MT (grey squares or column) and WT (black squares or column) as described in *Methods*. **a:** Glucose levels during clamp procedure. **b**–**c**: GIRs during the clamp procedure. **d:** Rd. **e:** Basal (filled columns) and during the clamp (striped columns) EGP. Glucose uptake into skeletal muscle (**f**), BAT (**g**) and WAT (**h**) during the clamp. Values are expressed as means ± SEM of determinations in at least 8 mice per group. $^{**}P < 0.01$, and $^{***}P < 0.001$ vs. WT.

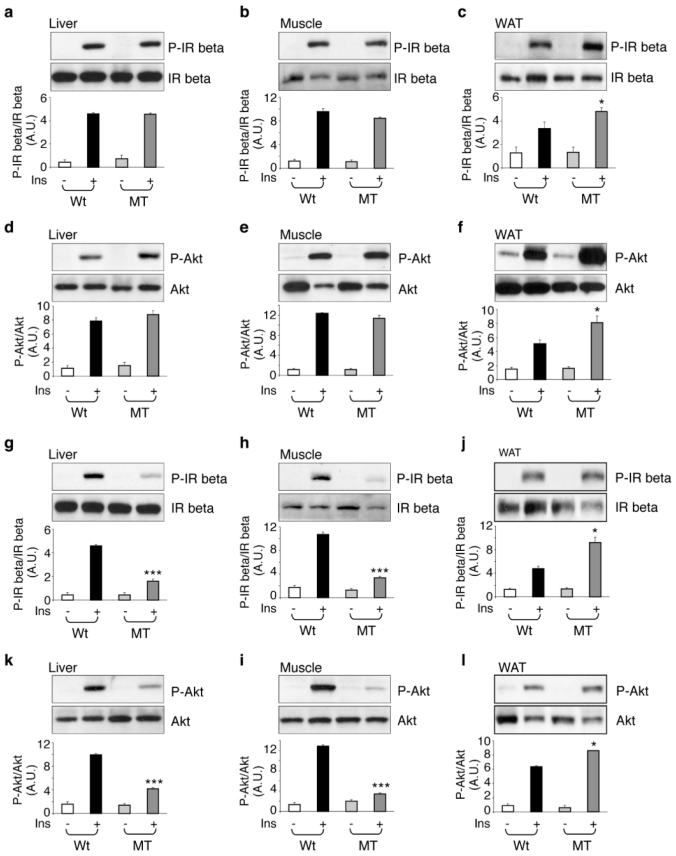

Fig. 3.

Insulin signaling in *Ankrd26* mutant mice. *In vivo* insulin-signaling in liver (**a**, **d**, **g** and **k**), tibialis skeletal muscle (Muscle; **b**, **e**, **h** and **i**) and epididymal WAT (**c**, **f**, **j** and **l**) of 2- (**a–f**), and 6- (**g–l**) month-old overnight fasted MT and WT mice i.p. injected (dark grey and black columns) or not (light grey and white columns) with insulin (Ins; 10 U•kg body weight^{-1}) for 10 min. Blots show the protein levels of total and tyrosine (Tyr-1150/1151) phosphorylated form of IR, and total and serine (Ser-473) phosphorylated form of Akt in these mice. Each autoradiographs shown on the top of graphics is representative of three independent experiments. Bars are expressed as means ± SEM in at least 6 mice per group. MT + Ins vs. WT + Ins. **P* < 0.05, and ****P* < 0.001.

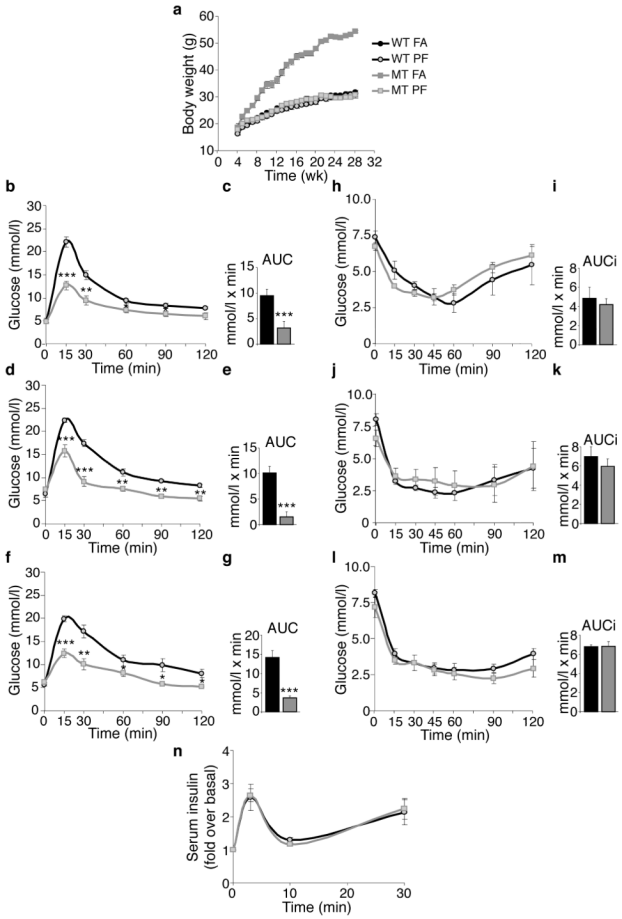

Fig. 4.

Body weight, glucose tolerance, insulin sensitivity and insulin secretion in pair fed *Ankrd26* mutant mice. **a:** Growth curve of MT (grey squares filled with dark and light grey) and WT (black circles filled with black and grey) mice under AL feeding regimen or subjected to a PF diet. GTT in 2- (**b**), 4- (**c**) and 6- (**d**) month-old PF MT and PF WT mice. Mean AUC of MT (grey column) and WT (black column). ITT in 2- (**e**), 4- (**f**) and 6- (**g**) month-old random fed PF MT and PF WT mice. Mean AUCi of MT (grey column) and WT (black column). For each experiment, values are expressed as means ± SEM of determinations in at least 6 mice per group. **h:** Glucose-induced insulin secretion in 6-month-old PF MT (grey squares filled with grey) and PF WT mice (black circles filled with grey). Data points represent the means ± SEM of determinations in 4 MT and 5 control mice. **P* < 0.05, ***P* < 0.01, and ****P* < 0.001 vs. PF WT.

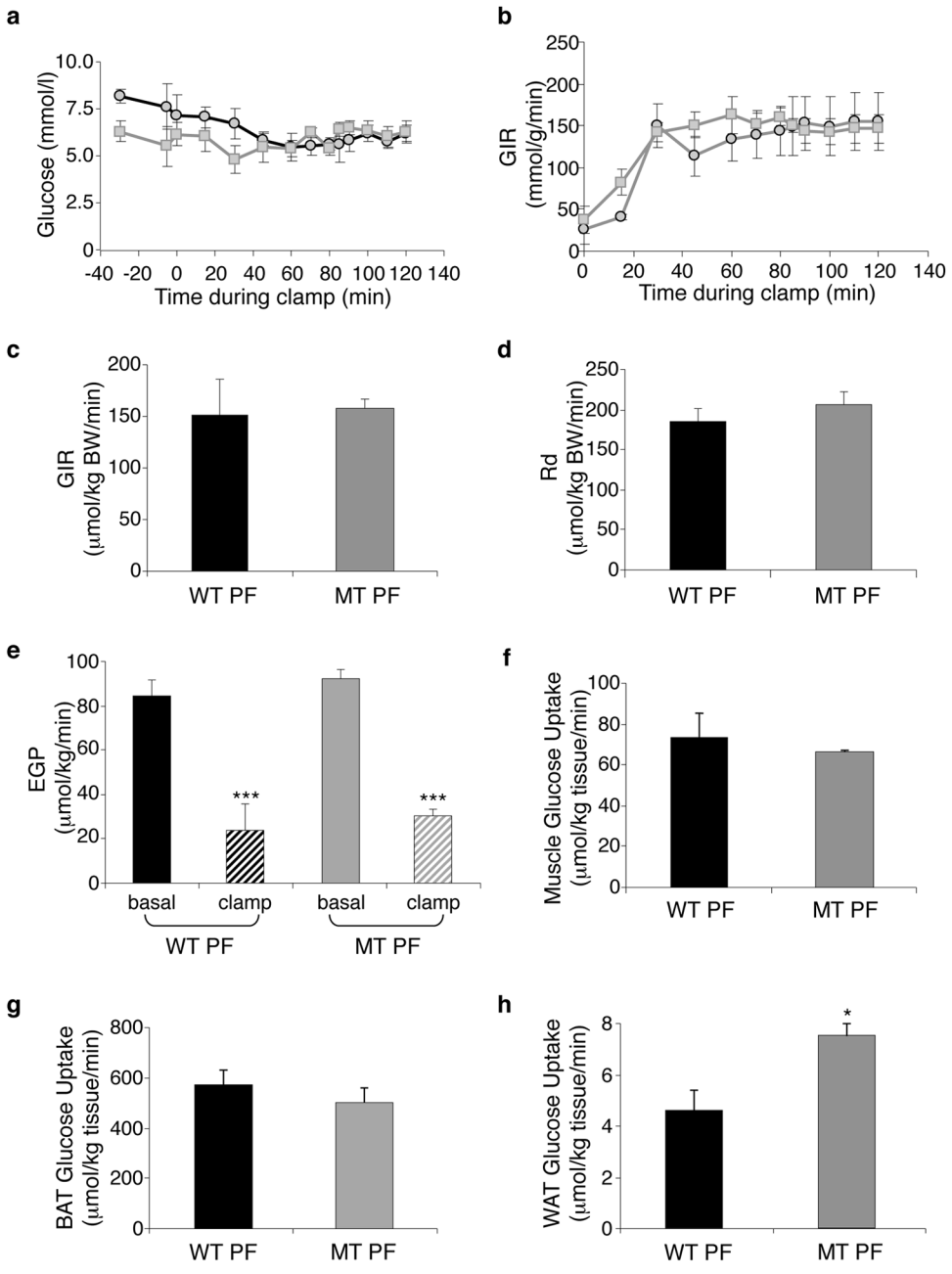

Fig. 5.

Hyperinsulinemic-euglycemic clamp studies in pair fed *Ankrd26* mutant mice. Hyperinsulinemic-euglycemic clamp studies were performed in 7-month-old MT (grey squares filled with light grey and grey column) and WT (black circles filled with grey and black column) mice upon PF. **a:** Glucose levels during clamp procedure. **b–c**) GIRs during the clamp procedure. **d:** Rd. **e:** Basal (filled columns) and during the clamp (striped columns) EGP. Glucose uptake into skeletal muscle (**f**), BAT (**g**) and WAT (**h**) during the clamp. Values are expressed as means ± SEM of determinations in at least 5 mice per group. **P* < 0.05, and ****P* < 0.001 vs. PF WT.

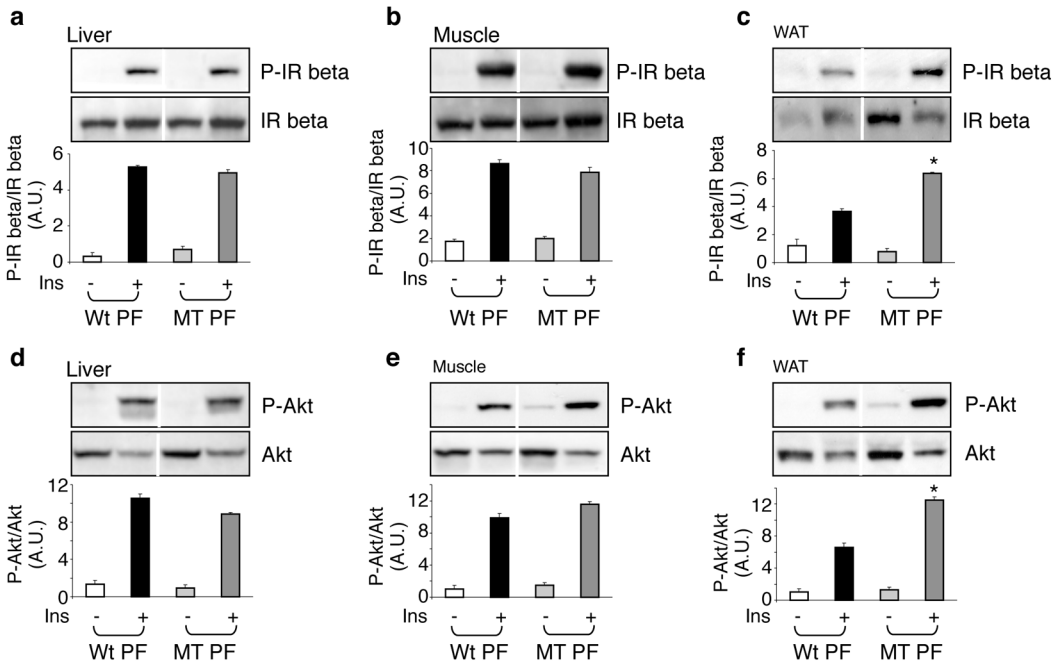

Fig. 6.

Insulin signaling in pair fed *Ankrd26* mutant mice. *In vivo* insulin-signaling in liver (**a** and **d**), tibialis skeletal muscle (muscle; **b** and **e**), and epididymal WAT (**c** and **f**) of 6-month-old overnight fasted MT and WT mice under PF feeding regimens i.p. injected (dark grey and black columns) or not (light grey and white columns) with insulin (Ins; 10 U•kg body weight^{-1}) for 10 min. Blots show the protein levels of total and tyrosine (Tyr-1150/1151) phosphorylated form of IR, and total and serine (Ser-473) phosphorylated form of Akt in these mice. Each autoradiographs shown on the top of graphics is representative of 3 independent experiments. Bars are expressed as means ± SEM in at least 6 mice per group. MT + Ins vs. WT + Ins. **P* < 0.05. The lanes were run on the same gel but were noncontiguous.

Table 1

Metabolic characteristics of *Ankrd26* mutant mice

	2 months		4 months		6 months	
Body characteristics	**WT**	**MT**	**WT**	**MT**	**WT**	**MT**
Body weight (g)	23.8 ± 0.5	30.3 ± 0.6^a	27.8 ± 0.5	40.5 ± 1.0^b	30.4 ± 0.9	48.1 ± 1.2^c
Body length (mm)	91.8 ± 0.4	98.5 ± 0.3^a	94.7 ± 0.4	100.5 ± 0.6^b	96.8 ± 0.4	105.0 ± 0.9^c
Metabolites	**WT**	**MT**	**WT**	**MT**	**WT**	**MT**
Fasting glucose (mmol/l)	4.4 ± 0.2	6.0 ± 0.4^a	7.1 ± 0.5	10.0 ± 0.3^b	95.6 ± 2.9	5.3 ± 0.3^c
Fed glucose (mmol/l)	6.3 ± 0.3	7.4 ± 0.4^a	8.1 ± 0.4	11.8 ± 1.1^b	6.8 ± 0.4	12.8 ± 1.1^c
Fasting insulin (pmol/l)	65.5 ± 6.1	137.2 ± 16.8^a	91.5 ± 10.6	202.8 ± 21.3^b	70.1 ± 6.1	364.4 ± 28.9^c
Fasting leptin (ng/ml)	2.60 ± 0.30	11.42 ± 1.80^a	5.58 ± 0.87	36.74 ± 3.46^b	7.25 ± 0.99	56.60 ± 8.79^c
Fasting NEFA (g/l)	0.19 ± 0.03	0.23 ± 0.02	0.30 ± 0.03	0.25 ± 0.02	0.33 ± 0.03	0.26 ± 0.02
Fasting Triacylglycerol (mmol/l)	51.6 ± 4.9	63.9 ± 6.4	49.7 ± 9.7	48.5 ± 5.9	54.3 ± 3.0	57.4 ± 1.3

Data are means ± SEM of determinations in at least 10 mice per group. Two-month-old

Ankrd26 MT mice (MT) vs. 2-month-old normal litter mates (WT);

$^a P < 0.001$. Four-month old MT vs. 4-month-old WT;

$^b P < 0.001$. Six-month-old MT vs. 6-month-old WT;

$^c P < 0.001$.

Table 2

Metabolic characteristics of pair fed *Ankrd26* mutant mice.

	2 months		4 months		6 months	
Body characteristics	**WT PF**	**MT PF**	**WT PF**	**MT PF**	**WT PF**	**MT PF**
Body weight (g)	20.1 ± 0.3	22.1 ± 0.5[a]	24.9 ± 0.5	27.8 ± 0.6[d]	29.1 ± 0.8	29.9 ± 0.5
Body length (mm)	91.6 ± 0.7	93.0 ± 0.3	94.3 ± 0.3	94.8 ± 0.4	96.5 ± 0.8	97.0 ± 0.3
Fat mass (%)	NA	NA	NA	NA	19.7 ± 2.5	20.1 ± 3.0
Lean mass (%)	NA	NA	NA	NA	76.6 ± 2.4	76.0 ± 3.0
Metabolites	**WT PF**	**MT PF**	**WT PF**	**MT PF**	**WT PF**	**MT PF**
Fasting glucose (mmol/l)	4.9 ± 0.3	4.8 ± 0.3	6.4 ± 0.6	6.5 ± 0.3	5.9 ± 0.7	6.2 ± 0.4
Fed glucose (mmol/l)	6.7 ± 0.7	6.3 ± 0.3	8.2 ± 0.5	7.5 ± 0.3	7.9 ± 0.2	7.2 ± 0.3
Fasting insulin (pmol/l)	61.0 ± 4.5	33.5 ± 4.5[b]	65.5 ± 28.9	61 ± 7.6	62.5 ± 19.8	54.9 ± 12.2
Fasting leptin (ng/ml)	2.79 ± 0.18	2.15 ± 0.23	3.82 ± 0.20	6.10 ± 0.86[c]	5.45 ± 1.35	6.67 ± 1,24
Fasting NEFA (g/l)	0.16 ± 0.01	0.11 ± 0.01[b]	0.39 ± 0.02	0.20 ± 0.02[e]	0.38 ± 0.01	0.23 ± 0.02[f]
Fasting Triacylglycerol (mmol/l)	75.2 ± 3.4	52.7 ± 19.0	62.6 ± 11.3	38.0 ± 6.8	53.5 ± 2.4	35.6 ± 2.8[f]

Data are means ± SEM of determinations in at least 5 mice per group. Two-month-old pair fed (PF) *Ankrd26* MT mice (MT) vs. 2-month-old PF normal litter mates (WT);

[a] $P < 0.05$, and

[b] $P < 0.01$. Four-month-old PF MT vs. 4-month-old PF WT;

[c] $P < 0.05$,

[d] $P < 0.01$, and

[e] $P < 0.001$. Six-month-old PF MT vs. 6-month-old PF WT;

[f] $P < 0.001$.