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# Severely Impaired Adipsin Expression in Genetic and Acquired Obesity

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**Adipsin, a serine protease homolog, is synthesized and secreted by adipose cells and is found in the bloodstream. The expression of adipsin messenger RNA (mRNA) and protein was analyzed in rodents during metabolic perturbations and in several experimental models of obesity. Adipsin mRNA abundance is increased in adipose tissue during fasting in normal rats and in diabetes due to streptozotocin-induced insulin deficiency. Adipsin mRNA abundance decreased during the continuous infusion of glucose, which induces a hyperglycemic, hyperinsulinemic state that is accompanied by an increased adipose mass; it is suppressed (>100-fold) in two strains of genetically obese mice (*db/db* and *ob/ob*), compared to their congenic counterparts, and is also reduced when obesity is induced chemically by injection of monosodium glutamate into newborn mice. Circulating adipsin protein is decreased in these animal models of obesity, as determined by immunoblotting with antisera to adipsin. Little change in adipsin expression is observed in a model of obesity obtained by pure overfeeding of normal rats (cafeteria model). These data suggest a possible role for adipsin in the above-mentioned disordered metabolic states, and raise the possibility that adipsin expression may be used to distinguish obesities that arise from certain genetic or metabolic defects from those that result from pure overfeeding.**

THE OBESITIES ARE A HETEROGENEOUS group of disorders characterized by increased adipose cell mass (*1*). Obesity may be viewed as a disorder of systemic energy balance, and molecular defects that contribute to the pathophysiology of the obese state may exist within any of the systems responsible for regulation of food intake and energy expenditure. One cellular site at which potential abnormalities might reside is the adipocyte. Physiologic experiments have suggested the existence of a putative fat cell–derived signal that might exert systemic effects on appetite control or energy expenditure (an adipostat) (*2, 3*). However, molecular evidence for such a factor has not been forthcoming, and suitable candidates for the role of a fat cell–derived systemic regulator have not been identified. Adipsin is a member of the serine protease gene family (*4*) whose principal site of synthesis is the adipose cell (*4–6*). This protein is secreted and is present in the peripheral circulation (*7*), and, as such, adipsin may be viewed as the first specific candidate for such a class of fat cell–derived regulatory molecules. As our initial assessment of this possibility, we evaluated adipsin messenger RNA (mRNA) expression and circulating adipsin protein of rodents in response to several nutritional and metabolic perturbations as well as in several syndromes of rodent obesity. We find that adipsin mRNA abundance is subject to physiological regulation, and that some (but not all) genetic and acquired obesity syndromes are associated with profoundly reduced expression of adipsin mRNA and circulating adipsin protein.

We first examined the regulation of several adipocyte genes in rat adipose cells and tissue during nutritional or hormonal manipulations that place the adipocyte into a lipolytic state or a state that favors lipid accumulation. Epididymal adipocyte mRNA was isolated and analyzed by Northern blotting (*8*). The mRNAs examined encode adipsin (*4, 5*), glycerophosphate dehydrogenase (GPD) (*5*), a key lipogenic enzyme, and aP2, a putative lipid-binding protein (*5, 9, 10*). These genes are all expressed in adipocytes in a differentiation-dependent manner.

A large increase in GPD mRNA occurred during the lipogenic stimulus of a 3-day intravenous glucose infusion (*11*) (Table 1); this observation is consistent with the function of GPD as a key regulated step of lipogenesis. The aP2 mRNA is also induced by glucose infusion but somewhat less dramatically than GPD. In contrast, glucose infusion suppressed adipsin mRNA. However, an increase in adipsin mRNA occurs when a lipolytic state is brought about by fasting, severe streptozotocin-induced diabetes (*12*), or mild diabetes induced by a partial pancreatectomy (*13*).

The expression of these genes was next examined in the fat tissue of animals in two distinct genetic models of obesity and obesity-linked diabetes: the *ob/ob* mouse and the *db/db* mouse (*14*), two strains that are homozygous-recessive at genetic loci residing on chromosomes 6 and 4, respectively (*14*) (Fig. 1). Both strains are characterized by massive obesity that becomes apparent by 2 weeks after birth (*14*). In addition to obesity, these animals exhibit hyperphagia, hyperglycemia, hyperinsulinemia, impaired thermogenesis, as well as a wide variety of other defects (*14*). At present, the nature of

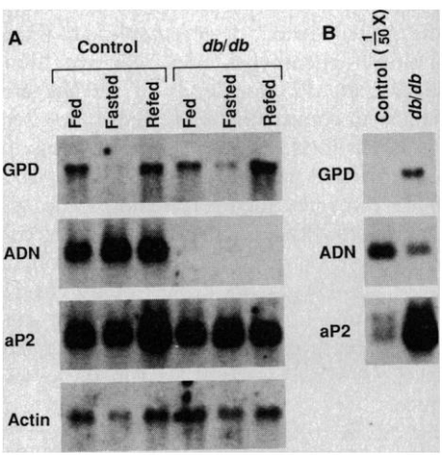


**Fig. 1.** (**A**) Specific mRNA levels in adipose tissue of *db/db* mice and controls. Male *db/db* mice 8 to 12 weeks of age (Jackson Laboratory) and control age-matched congenic C57BL/KSJ mice were allowed free access to standard laboratory diet (Fed), deprived of the diet for 3 days (Fasted), and refed for 3 days with the same diet after a 3-day fast (Refed). Total cellular RNA was extracted from epididymal fat pads, separated by electrophoresis, transferred to nylon filters, and probed with GPD, adipsin (ADN), aP2, and actin as described in the legend to Table 1. Pooled fat pads were taken from four to six animals in each treatment group. The same results were observed in two litters of *db/db* animals obtained at 6-month intervals. Each lane contains 5 μg of RNA. (**B**) In order to facilitate comparison of signal intensities for specific mRNAs in samples from control and *db/db* mice, the *db/db* RNA was separated by electrophoresis in 50-fold excess (5 μg) relative to that in the control lane (0.1 μg).

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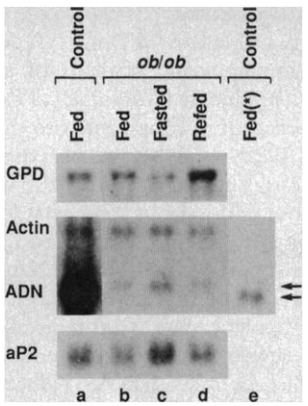


**Fig. 2.** Specific mRNA levels in adipose tissue of *ob/ob* mice and controls. Male *ob/ob* mice 8 to 12 weeks of age (Jackson Laboratory) and control age-matched congenic C57BL/6J were studied while being fed freely the standard laboratory diet (Fed) (lanes a, b, and e), after 3 days of deprivation of food (Fasted) (lane c), and after 3 days of refeeding after a 3-day fast (Refed) (lane d). Extraction of RNA from epididymal fat, electrophoresis, transfer, and probing were as described above. Lane e was exposed 1/50 the time lane a was exposed, to permit a more direct comparison of mRNA size in *ob/ob* and control. Arrows point to adipsin species in *ob/ob* and control mice. As in Fig. 1, the RNA was pooled from four to six animals in each treatment group, and the same results were seen in animals from a different litter.

the primary genetic defects that lead to these multiple phenotypes are unknown. For GPD and aP2, the levels of mRNA are similar in control and *db/db* mice given free access to food. Fasting caused a drop in GPD mRNA in both strains of mice, and both recovered upon refeeding. The response of GPD mRNA to fasting and feeding was thus similar in the control and obese mice (*15–17*). Levels of aP2 mRNA were very similar in the control and obese animals, except for a twofold induction in refed controls, which was not seen in the obese mice.

Adipsin mRNA levels were profoundly decreased (at least 100-fold) in the *db/db* mice when compared to their congenic controls in the fed state. Fasting of the obese mice, which should reverse any changes tightly linked to the hyperphagia of these animals, failed to substantially increase the adipsin mRNA level. Fasting of congenic controls caused a twofold increase in adipsin mRNA. This smaller increase compared to that in the rat is probably due to the greater general loss of mRNA during fasting in the mouse [see (*15–17*)]. To better examine the very weak adipsin signals observed in the *db/db* strain, we separated by electrophoresis the *db/db* mRNA next to 1/50 as much mRNA from the control mouse. When these fragments were blotted and hybridized to probes of high specific activity, these mRNAs appear to be the same size.

It is important to know whether this very large change in adipsin mRNA is specific to the *db/db* model or whether it can be observed in a genetically distinct strain of obese mice, *ob/ob*. It is evident that GPD and aP2 mRNA levels are similar in the two strains. However, adipsin mRNA is again severely reduced in *ob/ob* mice (by a factor of 100) (Fig. 2). Moreover, the residual adipsin mRNA in the *ob/ob* animals has a different size, 1.25 kb instead of 1.1 kb. These differences in size and quantity cannot be reversed by depriving the *ob/ob* animals of food for 3 days.

A question of central importance is whether these differences in mRNA are reflected in the amounts of adipsin protein. This protein can be measured by Western blotting with antisera to specific peptides of the adipsin molecule (*7*). This method shows that this protein is secreted from cultured adipocytes and can be found both in adipose tissue and in peripheral blood (*7*). Adipsin protein exists as a distinct band (38 kD) in adipose tissue extracts from both control strains congenic to the obese animals (Fig. 3, lanes a to c and g). This signal can be eliminated by absorption of antisera with its cognate peptide. In contrast, adipsin is virtually undetectable in extracts from the fat tissue of both the *db/db* and the *ob/ob* mice (lanes d to f and h to j).

Adipsin is readily detected in the serum of both control strains of mice as a broad band of 37 to 44 kD (Fig. 4) but is dramatically reduced in the serum of both strains of obese mice. Moreover, the proteins have a somewhat different pattern in gels, with both obese strains yielding a sharper band at approximately 40 kD compared to the broader signal in the control strains. Since the change in size from the 28-kD primary translation product to the protein secreted from cells is primarily due to glycosylation (*7*), the differences in adipsin mobility between the obese and normal animals may be due to altered (less heterogeneous) glycosylation.

To determine whether adipsin expression is impaired in an acquired syndrome of obesity, we chose the monosodium glutamate (MSG) model (*18*). Newborn mice injected subcutaneously with large amounts of MSG develop hypothalamic damage and obesity despite normal food intake (*18*). Impaired sympathetic nervous system activation of thermogenesis contributes to the adiposity of these animals (*19*). Two months after MSG treatment, at which point epididymal fat pad weight (which reflects overall obesity) was increased three times that of noninjected controls, adipsin mRNA expression was reduced in fat pads of MSG-treated animals (Fig. 5). Similarly, adipsin protein was markedly reduced in extracts from fat pads (Fig. 3) and in serum (Fig. 4) from MSG-treated animals. The electrophoretic mobilities of adipsin mRNA and protein were unchanged in this model (Figs. 3 to 5).

Since adipsin gene expression was re-

**Table 1.** Metabolic regulation of fat cell mRNA in the rat (signal intensity relative to controls). Four sets of male Sprague-Dawley rats were studied. In the first model, rats (200 g) were allowed free access to standard laboratory diet (fed controls) or deprived of the diet for 3 days (fasted). In the second model, 200-g rats were injected with 40 mg of streptozotocin per kilogram of body weight (*12*) and studied 3 weeks later. A control rat was age-matched and untreated. Blood glucose of the streptozotocin-treated animals was 450 mg/dl. In the third model, 200-g male rats were subjected to 90% surgical pancreatectomy or sham-operated. The pancreatectomized rats had blood glucose of 170 mg/dl. In the fourth model, rats (200 g) were infused with dextrose or saline (controls) for 48 hours as described (glucose infusion) (*11*). The dextrose-infused rats had blood glucose of 370 mg/dl (compared to 146 mg/dl for controls) and plasma insulin levels of 195 μU/ml (compared to 8 μU/ml for controls). RNA from individual rats was extracted from epididymal fat pads with guanidinium isothiocyanate and CsCl centrifugation (*8*), and 10-μg aliquots were separated by electrophoresis (*8*), blotted to nylon filters, and probed with complementary DNAs for GPD, adipsin (ADN), aP2, and actin (*30*). Radiolabeling with $^{32}P$ was by the random priming method (*31*). The hybridization and washing procedures were as described (*8*). Autoradiograms were scanned by laser densitometry, and all values were normalized to the signal obtained for actin mRNA. This normalization caused no more than a 30% change from basing the results per unit of total RNA. Similar results were obtained in three separate fasting experiments and two separate experiments involving the other manipulations. <25% Δ, less than 25% change; +, increased mRNA relative to controls; and −, decreased mRNA relative to controls.

| Treatment | mRNA | | |
|---|---|---|---|
| | GPD | ADN | aP2 |
| Catabolic | | | |
| Fasted | <25% Δ | 4.8-fold + | 40% + |
| Streptozotocin | <25% Δ | 3.9-fold + | <25% Δ |
| Pancreatectomy | <25% Δ | 90% + | <25% Δ |
| Anabolic | | | |
| Glucose infusion | 12.8-fold + | 65% − | 3.1-fold + |

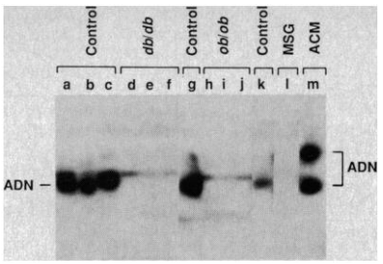


**Fig. 3.** Western blotting of adipose tissue extracts with adipsin antisera. Epididymal adipose tissue from animals described in Figs. 1, 2, and 5 was excised, and sonic extracts were prepared (*7*). Electrophoresis, immunoblotting, and probing with antisera to adipsin were as described (*7*). The antiserum used for this blot was raised against a 14–amino acid peptide (peptide 2) from adipsin and is described in the accompanying report (*7*). Lanes a to c (control) were from fed (a), 3-day fasted (b), and refed (c) controls congenic to *db*/*db* animals, and lanes d to f were from fed (d), fasted (e), and refed (f) *db*/*db* mice. Lane g is from an ad libitum–fed control mouse congenic to the *ob*/*ob*, and lanes h to j (*ob*/*ob*) are from fed (h), fasted (i), and refed (j) *ob*/*ob* mice. Lane k is from a pool of four freely fed control CD-1 mice (Charles River). Lane l (MSG) is from a pool of four CD-1 mice treated neonatally with subcutaneous injection of monosodium glutamate (3 mg per gram of body weight) and studied 12 weeks later at which time the epididymal fat pad weight was three times that of the nontreated control. Lane m is conditioned medium from cultured 3T3-F442A adipocytes (ACM) (*7*). ADN denotes the positions of the 37- and 44-kD forms of adipsin from conditioned medium. Tissue extracts contain adipsin of 37 to 38 kD (*11*). The narrow band above adipsin and detectable in the *db*/*db* and *ob*/*ob* lanes is an artifact of the immunoblot procedure and, unlike the adipsin band, is not eliminated by prior absorption of antisera with excess antigen peptide (*7*).

duced in two distinct genetic and one chemically induced model of rodent obesity, we next assessed adipsin gene expression in a model (the cafeteria-fed rat) that is viewed as being more representative of simple gluttony, without an underlying neuroendocrine or metabolic defect. In this model, rats are exposed to a frequently changed array of highly palatable foods, and under these conditions they typically ingest more calories than when exposed to laboratory pellets (*20*). We studied normal 8-week-old Sprague-Dawley rats, some of which were then "cafeteria-fed" for 12 weeks. The cafeteria-fed animals weighed 10% more than controls fed laboratory food, had plasma insulin levels that were two to three times as much as those of normal animals, and epididymal fat pads that were three times as heavy as those of controls. Brown fat, the most important site of the adaptive thermogenesis (*21*) that is known to occur in this model of overfeeding (*22*), was hypertrophied to twice that of controls. Two experiments with different animals were performed. Adipsin mRNA actually increased two to three times per unit total RNA in the obese animals as compared to the controls but was essentially identical when normalized to the actin signal (Fig. 5). In the second set of animals, a modest decrease (50%) in adipsin mRNA was observed relative to actin. No difference in the extent of obesity was apparent between these two sets of animals. Thus, the sharp decrease in adipsin mRNA (1/25 to 1/200 of controls) seen in the genetically and chemically induced obese models was not seen in the cafeteria-fed rats. Smaller quantitative changes resulting from these overfeeding protocols remain a possibility. It has not yet been possible to detect adipsin protein in rat serum or tissue extracts with the antisera prepared against peptides of mouse adipsin. Because the impaired adipsin expression was observed in three models of obesity in the mouse, whereas gluttonous obesity was studied in the rat, there might be concern that the relatively constant adipsin mRNA in the cafeteria-feeding model reflects a species difference. However, preliminary results show a large suppression of adipsin mRNA in the Koletsky strain of genetically obese (*fa*/*fa*) rat (*23*), a model that is similar in many ways to the genetically obese mice used here.

Our finding that adipsin mRNA expression in adipose cells is greatly reduced in both strains of genetically obese mice and in MSG-induced obesity represents one of the first examples of impaired gene expression associated with this disorder. Further, the severity of the defect in adipsin expression and the fact that it is not reversed after 3 days of caloric restriction indicate that this suppression is not obligatorily related to hyperphagia and its metabolic sequelae. This raises the possibility that adipsin deficiency, rather than simply being a consequence of obesity or hyperphagia, could play a pathogenetically important role in the genesis of some of the features of these animals. Consistent with this is the observation that an increase in adipsin mRNA appears to be a part of the catabolic state in normal rodents. It is intriguing that the *ob*/*ob* mice express an adipsin mRNA that is altered in size compared to both its congenic normal strain and to other obese mice examined.

The variations of adipsin mRNA and protein in the animal models suggests that the expression of this gene reflects some basic differences in these obese states. The clearest difference that distinguishes the *db*/*db*, *ob*/*ob*, and MSG models, from the cafeteria-fed animals is that the former have underlying metabolic defects that lead to obesity whereas the latter become obese by simple gluttony. Physiologically the most obvious correlate of these categories relates to energy utilization and, especially to diet-induced thermogenesis. This component of overall heat production (and oxygen consumption) in mammals appears to serve as a physiological defense against excessive weight gain (*24*). Because obesity is a disorder of energy balance, the status of diet-induced thermogenesis has been assessed in many models of obesity. It is established that cafeteria-fed animals respond to overfeeding with increased sympathetic nervous system activity and, as a consequence, increased thermogenesis (heat production) (*24*). This adaptive increase in (diet-induced) thermogenesis in the cafeteria-fed rat is in contrast to the three other models of obesity, in which affected animals display defective thermogenesis. Whereas cafeteria-fed rats become obese despite an adaptive increase in energy expenditure, the obesity of the three other models is believed to be largely a result of impaired energy expenditure (*19, 21*). Adipsin expression may be linked to the rate of thermogenesis, or it may be associated with one of the hormonal or neuroendocrine pathways that control or are affected by the thermogenic process.

Since adipsin expression is reduced in the glucose-infused rat, in which insulin levels are high, and in *ob*/*ob* and *db*/*db* mice, which are also extremely hyperinsulinemic, insulin must be viewed as a possible mediator of

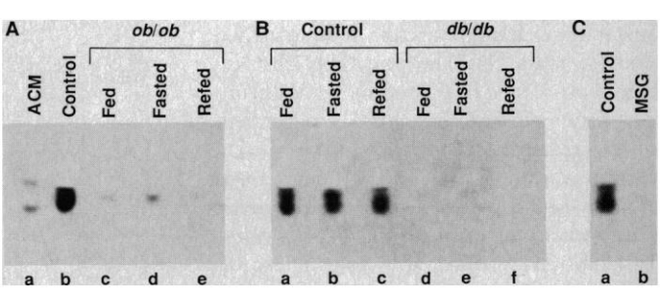


**Fig. 4.** Immunoblot of sera from normal and obese mice. Each serum sample (5 μl) [pooled from four to six animals for *ob*/*ob* (**A**) and *db*/*db* (**B**); individual mice for MSG (**C**)] was separated by electrophoresis, immunoblotted, and probed with antiserum as described in the legend to Fig. 3. Animals were the same as those described in Figs. 1, 2, 3, and 5. Identical results were obtained with two different sets of *db*/*db* and *ob*/*ob* mice. For the MSG experiment, eight treated mice were examined and a sharp reduction in adipsin level was shown in each MSG-treated animal compared to the same number of nontreated controls.

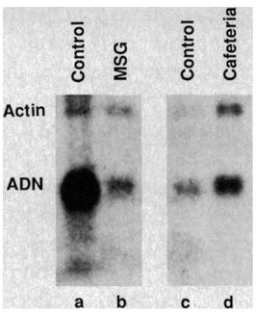


**Fig. 5.** Adipsin mRNA levels in obesity due to MSG injection or cafeteria-feeding. Northern blots were performed with 10 μg of RNA from adipose tissue of control mice or mice with MSG-induced obesity [legend to Fig. 3 and (*18*)], and control rat or rat exposed to cafeteria-feeding protocol (*20*). Data from mice are derived from fat pooled from four to six animals, whereas data from rats were obtained from two independent sets of paired, individual animals, as described in text.

reduced adipsin expression in these models. Several facts lead us to believe that insulin is not the sole factor regulating adipsin expression in these animals. First, 3 days of fasting in *ob/ob* and *db/db* mice do little to reverse the defect in adipsin expression, although circulating insulin levels are reduced during this period. Second, our findings of discordant adipsin expression in MSG-treated and cafeteria-fed animals cannot be explained by reference to insulin levels alone, as circulating insulin levels are normal or minimally increased in the former (*18*) and moderately increased in the latter (2.5 times as much as the control levels in the animals studied here). Thus, although insulin is likely to be a factor in the control of adipsin expression, other hormones or factors are likely to be involved in the regulation of this gene in obesity.

If the pattern of altered adipsin expression that we have found in rodents is also observed in obese humans, measurement of circulating adipsin levels could serve as a highly useful marker for characterizing obese patients. There has been considerable interest in the possibility that (as in rodents) a subset of obese patients have genetic or metabolic defects that result in an increased adipose mass (*1*). The possibility that reduced expression of the thermogenically important membrane $Na^+$- and $K^+$-dependent adenosinetriphosphatase might be such a marker was proposed; however, neither this nor any other marker defines unequivocally pathogenetically distinct subgroups of obese patients (*25*). Conceivably adipsin expression may prove useful as such a marker. Further studies in laboratory animals and human populations will be required to ascertain whether the level of adipsin expression defines a useful distinction between groups of obese patients.

What are the biochemical functions of adipsin protein? This circulating serine protease homolog could be an activator (or inactivator) or a hormone-signaling system or could itself be a novel fat-derived hormone or growth factor. There are precedents for circulating proteases subserving each of these roles. Thus, renin acts on a circulating substrate to generate the hormone angiotensin (*26*), and thrombin, in addition to its role as a protease in the clotting cascade, binds to specific platelet receptors (*27*), which are coupled to the activation of phosphatidylinositol turnover (*28*). Although we cannot yet assign a specific role to adipsin, we suspect that one of these paradigms will ultimately be shown to apply.

Of equal importance will be a determination of the physiological systems influenced by adipsin. As discussed above, adipsin mRNA is induced in several catabolic states. The most obvious response of adipose cells to a catabolic stimulus is a decrease in lipogenesis and an increase in lipolysis, two processes that are regulated through the interplay of numerous hormonal, metabolic, and neural factors. Adipsin could play a role in some aspect of the regulation of lipolysis, lipogenesis, or both. The decrease in adipsin expression observed in obese states is fully consistent with this hypothesis but also highlights possible systemic roles for this protein in the regulation of appetite or energy expenditure, or both. Clearly, these alternatives are not mutually exclusive. A link between pathways of lipolysis and thermogenesis has been previously suggested by studies that indicate that a large portion of the calories utilized in cold-induced nonshivering thermogenesis are derived through mobilization of body adipose stores (*29*). Clarification of these important physiological issues will require in vitro and in vivo studies with adipsin protein.

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