ducted similar experiments in which the exposure times of the frames were 100 and 200 msec, conditions that are known to produce optimal LR motion (*8*). The results of these experiments were not significantly different from those of experiments that used 48 msec per frame. These results suggest that the crucial parameter is the displacement of corresponding dots rather than temporal factors.

It was of interest to examine the role of the possible components of apparent motion in the motion detection process. If motion is not registered directly somehow, it could be inferred from the disappearance of the dot, its subsequent reappearance, and the spatio-temporal relations between these events. We performed several experiments to gain more insight concerning the detection of these events. We first examined the detection of an appearing and a disappearing dot, where other dots in the array remained stationary, and then the detection of motion among other appearing or disappearing dots.

In the disappearance detection test, one of the dots disappeared in the second frame in half the trials. In the appearance detection test, a new dot appeared in the second frame in half the trials. Experiments were done with the array sizes used previously (25 to 100 dots, same separation between dots). Results for the detection of a disappearing dot and that of an appearing dot are shown in Fig. 1. The detection of the appearance and of the disappearance of a single dot in a stationary array were clearly performed in parallel.

We further explored the relations between the detection of a disappearing dot and an appearing dot and the detection of motion. We tested the detection of a moving dot among appearing or disappearing background dots. The experiments were similar to those described above. The arrays subtended 11 × 11 degrees (64 dots). A test dot moved in half the trials while in the background a varying number of dots either appeared or disappeared among other stationary dots. The results show that the detection of motion among a varying number of either disappearing or appearing dots was processed in parallel only when SR motion was used. For LR motion the results were qualitatively different and indicative of a serial process (Fig. 3).

The results also show no interference of the appearing or disappearing dots with the detection of SR motion. In contrast, LR motion detection was affected by these distractors. Thus the detection of the direction of LR motion may involve the conjunction of disappearance and appearance detection. Although appearance and disappearance by themselves can be detected in parallel, their spatio-temporal conjunction may require, like various other conjunction tasks (*3*), a serial scan.

When exposure time was limited to 16 msec per frame, the detection of disappearance and appearance was no longer possible. As expected, the direction of LR motion under these conditions was also impossible to resolve (performance showed about 60% correct responses). Detection of the direction of SR motion remained unchanged. Averaged results of two subjects are plotted in Fig. 2 (dotted line). The detection of the direction of SR motion was clearly performed in parallel and independently at each location, implying the existence of an array of specialized detectors for the detection of the direction of SR, but not LR, motion.

It appears that, during the early stage of perception, the detection of the direction of motion is performed for SR motion only by a fast, parallel (preattentive) process. The detection of the direction of LR motion requires a serial search (an attentive process) and probably combines the detection of the disappearance and the reappearance of the object with additional spatio-temporal information. It is surprising to find such a serial, and relatively slow, motion detection system, since for many tasks motion must be computed relatively quickly. Such a system implies a limit on motion perception when LR motion is involved. Certain visual processes, which use the same information as LR motion (appearance and disappearance, for example), may have access to this information in a parallel fashion. For example, Ramachandran and Anstis (*9*) have reported an experiment that involved a global perception of multiple moving targets under LR conditions. It is not clear, however, whether this perception required the simultaneous parallel motion processing of the individual dots. It would be of interest to examine other perceptual tasks that rely on motion assignments, for example, whether fast global percepts could be obtained from local motion computations as in the "structure from motion" problem (*2*) when LR motion is used.

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Adipsin: A Circulating Serine Protease Homolog Secreted by Adipose Tissue and Sciatic Nerve

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Adipsin is a serine protease homolog whose primary structure was predicted from the nucleotide sequence of a differentiation-dependent adipocyte messenger RNA. Immunoblots probed with antisera to synthetic peptides identify two forms of adipsin that are synthesized and secreted by 3T3 adipocytes. These proteins of 44 and 37 kilodaltons are converted to 25.5 kilodaltons by enzymatic deglycosylation. Although adipsin is principally synthesized in adipose tissue, it is also produced by sciatic nerve and is found in the bloodstream. Because of the apparent restriction of adipsin synthesis to tissues highly active in lipid metabolism, its presence in serum, and its modulation in altered metabolic states, this molecule may play a previously unrecognized role in systemic lipid metabolism or energy balance.

DISORDERS THAT INVOLVE ADIPOSE tissue, such as obesity, are common and represent significant sources of mobidity (*1*). Intensive studies of adipocyte biochemistry over the past 20 years have revealed much about the hormonal control of carbohydrate and lipid metabolism. Despite these informative studies, our understanding of how adipocyte differentiation and metabolism are regulated is incomplete. New and potentially important gene products of the adipocyte have been identified by isolating complementary DNA (cDNA) clones corresponding to messenger RNAs (mRNAs) that are specifically induced during adipocyte differentiation (*2–4*). One

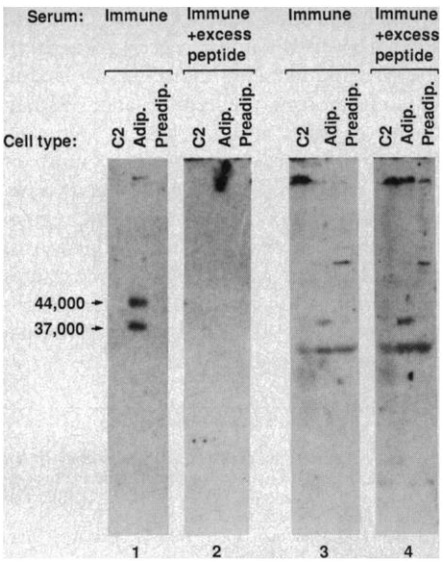

Fig. 1. Identification and localization of adipsin by immunoblotting. Secreted proteins (panels 1 and 2) were collected for 15 to 18 hours in Dulbecco's modified Eagle's medium without serum supplement. Sonic extracts were prepared as described (*17*). The lanes of secreted proteins contained 7.0, 5.4, and 3.2 μg of protein from 3T3-C2 (C2), and 3T3-F442A adipocytes (Adip.), and preadipocytes (Preadip.) (*11, 18*), corresponding to an equal fraction (1/25) of a confluent 100-mm culture. The lanes containing cell extracts (panels 3 and 4) were loaded to contain one-tenth of the total extract protein per dish; the amounts are 135, 160, and 80 μg for C2, adipocyte, and preadipocyte extracts. SDS-PAGE was performed on 10% acrylamide gels (*19*). Proteins were transferred to nitrocellulose (*20*), and the blots were probed with antisera to peptide 2 (*10*) and ^{125}I-labeled protein A (New England Nuclear). For the panels labeled "Immune + excess peptide," the serum (150 μl) was incubated with 225 μg of peptide 2 for 1 hour before the blots were probed.

such mRNA, originally called 28K and now referred to as adipsin (*5*), encodes a protein of 28 kD (*2*). This mRNA is expressed abundantly in mouse fat and either is undetectable or is expressed at much lower levels in other tissues (*6, 7*). The encoded protein shares sequence homology with the broad family of serine proteases. Adipsin is likely to be an active protease since it contains appropriately positioned histidine, aspartic acid, and serine residues required for catalytic activity as well as a cleavage site for activation of the zymogen. Like many other serine proteases, the deduced amino-terminal residues of adipsin appear to comprise a signal sequence (*6*). In the present study we have studied the biosynthesis, modification, and localization of adipsin protein in cultured adipocytes and normal mouse tissues.

To identify the protein or proteins encoded by adipsin RNA, we performed immunoblotting with serum directed against synthetic peptides corresponding to regions of adipsin deduced from the nucleotide sequence. Segments that show extensive primary sequence homology with other serine proteases were avoided to reduce the possibility of cross-reaction (*8, 9*). Peptides corresponding to the carboxyl terminus, amino acids 250 to 259 (peptide 1), and a putative external loop, amino acids 85 to 97 (peptide 2), were used to raise antisera (*10*). Adipocytes secrete proteins of 44 and 37 kD that are recognized by antisera to peptide 2 (Fig. 1, panel 1); these proteins are not present in preadipocytes or 3T3-C2 fibroblasts (*11*). The interaction between the antisera and the 44- and 37-kD proteins is specific; when these antisera are first adsorbed with an excess of soluble peptide, the 44- and 37-kD proteins are not detected (Fig. 1, panel 2). Antisera to peptide 1 also reacted with the 44- and 37-kD proteins secreted by adipocytes. We now refer to these polypeptides as adipsin.

Cultured cell extracts contain no proteins that react specifically with either of the two antipeptide antisera. The signals observed with immune serum (Fig. 1, panel 3), which included that of a 38-kD protein detected only in adipocytes, were not eliminated by competition with excess peptide (Fig. 1, panel 4). Preimmune serum does not react with either the 44- and 37-kD proteins secreted by adipocytes or the nonspecific bands detected in the cell extract blots.

The 44- and 37-kD adipsin molecules are much larger than the 28-kD protein deduced from the nucleotide sequence. Forms of adipsin generated by cleaving the signal peptide and activating the zymogen are predicted to be 26 and 25.5 kD, respectively (*6*). To establish (i) whether glycosylation accounts for the difference between the observed and deduced molecular weights of adipsin and (ii) whether removal of carbohydrate from the 44- and 37-kD forms will give rise to one lower molecular weight protein or two proteins still differing by 7 kD, we enzymatically deglycosylated adipsin, and analyzed the products by Western blotting. Upon digestion with endoglycosidase F, the 44- and 37-kD proteins were converted to a 25.5-kD protein (Fig. 2). Thus, the 44- and 37-kD molecules apparently differ from one another and from the deduced molecular mass of adipsin as a result of glycosylation.

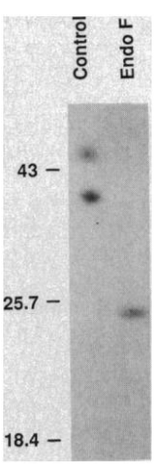

Fig. 2. Enzymatic deglycosylation of adipsin. Proteins secreted by adipocytes were concentrated to 1/13 their volume and dialyzed against 50 m*M* sodium phosphate, *p*H 7.4, 30 m*M* EDTA. Samples were adjusted to contain 0.1% SDS and 1.0% β-mercaptoethanol and were digested with endoglycosidase F (1.5 U/ml, Boehringer Mannheim) overnight at 37°C. Control samples were incubated without the addition of enzyme. The products were analyzed on Western blots probed with antiserum to peptide 2. The signal from the 25.5-kD protein is eliminated by competition with an excess of soluble peptide and is unaltered by digestion with three times as much enzyme.

To verify that the production of adipsin by cultured adipocytes accurately reflects the biosynthesis of this protein in animal tissue, we examined explant cultures of mouse epididymal adipose tissue for the synthesis and secretion of adipsin. We also tested sciatic nerve explants for adipsin synthesis, because the Schwann cells of myelinated nerve are very active in lipid metabolism, and another adipocyte protein, aP2, has been shown to be homologous to the Schwann cell protein myelin P2 (*3, 12*). The results of immunoprecipitations show that both adipose tissue and sciatic nerve synthesize adipsin in approximately equal amounts per unit of secreted radiolabeled protein (Fig. 3). The major form of the protein has a slightly lower mobility in SDS-PAGE (polyacrylamide gel electrophoresis) than the 37-kD species from 3T3 adipocytes.

Tissue extracts were tested to determine whether all of the adipsin synthesized in explant cultures is secreted. Adipsin was undetectable in sciatic nerve extracts and was present at a very low level (< 0.5% of the secreted adipsin) in sonic extracts of adipose tissue, which suggest that, as is the case with cultured 3T3 adipocytes, adipsin is efficiently secreted. Muscle explants were also tested for expression of adipsin protein, since this tissue has been reported to contain adipsin RNA at a level about one-fifth that in 3T3 adipocytes (*7*). Adipsin was undetectable in either supernates or sonic extracts of the muscle explant cultures. However, interpretation of this result is complicated by the relatively inefficient labeling of proteins in muscle explant cultures compared to those of sciatic nerve and adipose tissue (*13*).

The synthesis and secretion of adipsin by sciatic nerve and the inability to detect the protein from muscle led us to examine total RNA for sequences homologous to adipsin.

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From Northern blotting and densitometry, we estimate the relative abundances of adipsin mRNA per unit of total RNA as follows: adipose tissue defined as 100%, sciatic nerve 38%, and muscle 3% (*14*).

Since adipsin is mostly or entirely secreted from its demonstratable sites of synthesis, we have investigated whether this protein is largely retained in the adipose tissue or whether it can be detected in the peripheral circulation. Equivalent amounts of protein from adipose tissue and serum were analyzed by immunoblotting. Adipose tissue extracts yielded a distinct band of approximately 37 kD (Fig. 4, lanes b and c), whereas a broad, somewhat darker band of 37 to 44 kD is seen in the serum sample (lane a). These signals were obliterated by prior adsorption of antisera with excess peptide (Fig. 4, lanes d and e). Since we estimate that a normal (nonobese) mouse contains approximately 15 to 75 times as much serum protein as adipose tissue protein, there is considerably more adipsin in serum than in fat tissue.

Our results indicate that adipsin is synthesized and secreted by cultured fat cells, epididymal fat tissue, and sciatic nerve. Whether there is synthesis by muscle is ambiguous, but synthesis at a low level cannot be ruled out (*14*). Because of the relatively high level of adipsin mRNA and protein synthesis in fat and the size of the adipose depot, adipose tissue is almost certainly the principal source of this protein. The absence of intracellular adipsin suggests that this protein is secreted constitutively as opposed to by a pathway involving vesicular storage and release upon stimulation. Adipsin is highly glycosylated, and most or all of the carbohydrate can be removed with endoglycosidase F. This finding suggests *N*-linked glycosylation and is consistent with the presence of five potential *N*-linked glycosylation sites in the amino acid sequence. Other circulating serine proteases are also known to be extensively glycosylated (*8*).

The fact that adipsin is efficiently secreted and is present in the bloodstream places it within an extremely limited but important group of adipocyte proteins. Adipsin could act locally to influence extracellular lipoprotein or lipid metabolism. However, the abundance of this protein in serum requires us to consider a possible systemic role in the regulation of adipocyte function or overall energy balance. The existence of a feedback control loop for energy metabolism that involves a systemic signal from adipocytes (for example, an adipostat) has been suggested (*15*). However, experimental evidence for the existence of a signaling molecule that is: (i) relatively specific for fat cells, (ii) responsive to nutritional and hormonal stimuli, and (iii) secreted into the blood has been previously lacking. Here we have shown that adipsin has two of these properties, that adipose tissue is the primary site of synthesis and that the protein is secreted into the blood. In the accompanying report (*16*), we provide evidence that adipsin mRNA and protein levels are modulated in a consistent manner in response to changes in the metabolic state of rodents. Thus, adipsin meets the initial criteria required of the systemic signaling molecule involved in lipid metabolism or energy balance. Adding further credence to this possibility is the fact that this protein belongs to a family of enzymes involved in other systemic functions such as blood clotting and the regulation of blood pressure. The production of this protein in high-level expression systems will permit a direct experimental approach to the study of adipsin function in normal and diseased metabolic states.

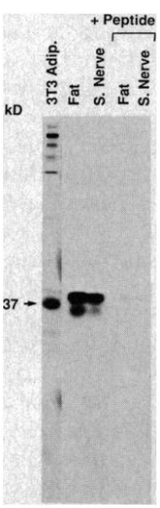

Fig. 3. Synthesis of adipsin by explant cultures of adipose tissue and sciatic nerve. Explant cultures of epididymal adipose tissue and sciatic nerve were prepared and labeled with [^{35}S]methionine (New England Nuclear, > 800 Ci/mmol) at 1 mCi/ml (*21*). Supernatants from adipose tissue (Fat) and sciatic nerve (S. nerve) containing 4.0×10^5 trichloroacetic acid–precipitable counts per minute and a 3T3 adipocyte control (3T3 Adip.) were immunoprecipitated essentially as described (*22*) with antiserum to peptide 1, with (+ Peptide) and without prior incubation of the serum with an excess of peptide 1. Antiserum to peptide 1 does not react strongly with the 44-kD form of adipsin. However, in adipose tissue the predominant form of adipsin appears to be ~37 kD (see Fig. 4).

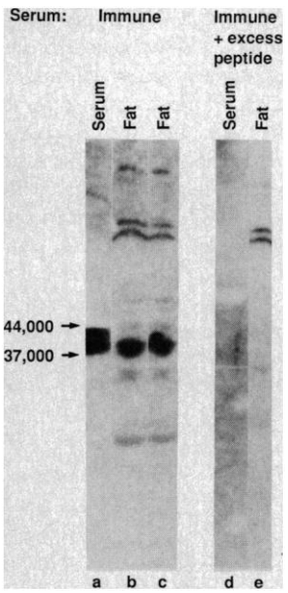

Fig. 4. Immunoblots showing adipsin in mouse serum and adipose tissue. Serum protein, 250 μg (lanes a and d), and adipose tissue protein (Fat), 250 μg (lane b), and 125 μg (lanes c and e), were analyzed by Western blotting with antiserum to peptide 2 as described in the legend to Fig. 1. The serum and adipose tissue were from male C57BL/6J mice. Tissue extract was prepared by sonicating 1 g of epididymal fat in 1 ml of 10 m*M* tris-HCl, *p*H 7.5, 1 m*M* EDTA and was clarified by centrifugation at 10,000*g* for 10 minutes.

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13. Extracts of muscle contained 700 counts per minute per microgram of protein, whereas those of sciatic nerve and adipose tissue contained 1.3×10^4 to 4.0×10^4 counts per minute per microgram of protein.
14. Northern blotting was as described in (*5*). Skeletal muscle RNA was prepared from thigh muscle from which the largest segment of the sciatic nerve had been removed. We have been unable to detect adipsin mRNA from C2, a myogenic cell line that expresses high levels of muscle-specific markers. The abundance of adipsin mRNA in sciatic nerve argues against the small number of adipocytes within the nerve sheath as the source of this mRNA. Instead, Schwann cells may be responsible for adipsin synthesis since nerve tissue lacking these cells (brain) does not express adipsin mRNA (*6*).
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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

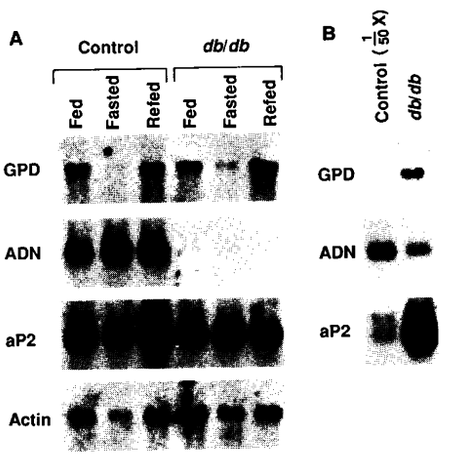

Fig. 1. (**A**) Specific mRNA levels in adipose tissue of *db/db* mice and controls. Male *db/db* mice 8 to 12 weeks of age (Jackson Laboratory) and control age-matched congenic C57BL/KSJ mice were allowed free access to standard laboratory diet (Fed), deprived of the diet for 3 days (Fasted), and refed 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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