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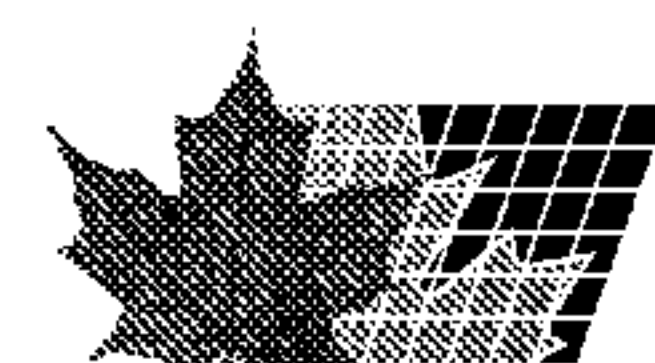
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(54) Title: PERILIPIN AS A TARGET FOR REGULATION OF BODY WEIGHT, MUSCLE MASS AND DIABETES

(57) **Abrégé/Abstract:**

The present invention relates to screening assays to identify compounds that modulate the activity or expression of perilipin. The present invention also relates to methods and therapeutic compositions for the prevention or treatment of body weight disorders or diabetes comprising administering to subjects compounds that modulate the activity or expression of perilipin. In one aspect, the invention of perilipin in order to treat and/or prevent obesity or diabetes, and to enhance lipid metabolism and muscle mass. In another aspect, the invention relates to methods and compositions that agonize the activity of perilipin in order to enhance lipid accumulation in a subject in order to increase weight gain.



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(54) Title: PERILIPIN AS A TARGET FOR REGULATION OF BODY WEIGHT, MUSCLE MASS AND DIABETES

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PERILIPIN AS A TARGET FOR REGULATION OF BODY WEIGHT, MUSCLE MASS AND DIABETES

5 This application is entitled to and claims priority benefit to U.S. provisional application Serial No. 60/238,272, the entire contents of which are incorporated herein by reference.

This invention was made with government support under grant number HL-51586
10 awarded by the National Institutes of Health. The government has certain rights in the invention.

1. INTRODUCTION

The present invention relates to a novel target, perilipin, for the regulation of lipid
15 homeostasis, muscle mass and body weight. The present invention relates to screening assays to identify compounds that modulate the activity or expression of the novel target of the invention, perilipin. The present invention also relates to methods and therapeutic compositions for the treatment of body weight disorders comprising administering to subjects compounds that modulate the activity or expression of perilipin. In one aspect, the
20 invention relates to methods and compositions that antagonize the activity or expression of perilipin in order to treat and/or prevent obesity or diabetes, and to enhance lipid metabolism and muscle mass. In another aspect, the invention relates to methods and compositions that agonize the activity of perilipin in order to enhance lipid accumulation in a subject in order to increase weight gain.

25

2. BACKGROUND OF THE INVENTION

In higher organisms, the primary function of the adipocyte is to store excess energy as triacylglycerol in the intracellular lipid droplet and to release the energy as fatty acids in time of need (Yeaman et al., 1990, Biochim. Biophys. Acta 1052: 128-132). In contrast to
30 the wealth of information on the metabolism of carbohydrate stores, in particular glycogen, the details of the mechanism whereby the lipid stores are packaged and hydrolysed are unknown. The rate limiting step of lipolysis, the process by which triacylglycerols are hydrolyzed to fatty acids and glycerol, is catalysed by hormone-sensitive lipase (HSL). Hormonal stimulation of HSL activity is associated with the translocation of the enzyme to
35 the surface of the lipid storage droplets. Perilipin is an abundant adipocyte protein that exists in three isoforms (A, B and C), which are products of differentially spliced transcripts

from a single gene. It has been postulated that under the basal state perilipin coats the lipid droplet and somehow interferes with HSL gaining access to the droplet (Londos et al., 1995, Biochem. Soc. Trans. 23: 611-615; Londos et al., 1999, Semin. Cell. Dev. Biol. 10: 51-58; Souza et al., 1998, J. Biol. Chem. 273: 24665-24669). Lipolytic activation of adipocytes is associated with protein kinase A-mediated phosphorylation of perilipin which induces a
5 change in the lipid droplet surface and the appearance of lipid microdroplets, allowing access of HSL to core lipids (Londos et al., 1999, Semin. Cell. Dev. Biol. 10: 51-58). There is however, much uncertainty on the role of perilipin in lipid homeostasis and energy metabolism *in vivo*.

10 3. SUMMARY OF THE INVENTION

The present invention relates to a perilipin as a target for the regulation of lipid homeostasis and the regulation of muscle mass and weight gain. The present invention encompasses screening assays to identify those compounds that would modulate the activity and/or expression of the perilipin protein, as a means of identifying compounds that would
15 be useful for the treatment of disorders related to body weight and/or inappropriate regulation of lipid metabolism. The invention also encompasses pharmaceutical compositions comprising compounds which modulate the activity and/or expression of the perilipin protein for the treatment of disorders related to body weight and/or inappropriate regulation of lipid metabolism.

20 The present invention is based, in part, on the Applicant's discovery of the critical role that the perilipin protein plays in lipid homeostasis, muscle mass and energy metabolism *in vivo*. In the examples provided *infra*, it is shown that when the perilipin gene is disrupted in mice (*plin*^{-/-} mice), it results in animals which exhibit constitutively activated hormone sensitive lipase. The *plin*^{-/-} mice may consume more food, but maintain a normal
25 body weight, as compared to control mice. The *plin*^{-/-} mice exhibit elevated basal lipolysis, increased metabolism and demonstrate resistance to diet induced obesity. Further, *plin*^{-/-} mice exhibit increased muscle mass in the absence of exercise. These results demonstrate that perilipin is a target for regulating lipolysis and energy balance and anti-obesity medications.

30 The present invention provides screening assays to identify compounds that modulate the activity, expression, and/or phosphorylated state of the perilipin protein. In particular, the present invention provides *in vitro* assays to identify perilipin-binding compounds using recombinantly expressed perilipin, cells endogenously expressing one or more perilipin isoforms (*e.g.*, adipocytes), or perilipin-transfected cell lines. The perilipin-
35 transected cell lines may further comprise a reporter gene whose level of expression is regulated by perilipin.

The present invention provides methods of screening for an agent that interacts with a perilipin isoform or a fragment thereof, comprising: (a) contacting a perilipin isoform or a fragment thereof with a candidate agent; and (b) determining whether or not the candidate agent interacts with the perilipin isoform or fragment thereof. In accordance with these methods, the perilipin isoform or fragment thereof may be endogenously expressed by cells
5 such as, *e.g.*, steroidogenic cells or adipocytes, or cells may be genetically engineered to express the perilipin isoform or fragment thereof.

The present invention also provides methods of screening for an agent that modulates the expression of a perilipin isoform, comprising: (a) contacting a first population of cells expressing the perilipin isoform with a candidate agent; (b) contacting a
10 second population of cells expressing said perilipin isoform with a control agent; and (c) comparing the level of said perilipin isoform or mRNA encoding said perilipin isoform in the first and second populations of cells. An agonist of perilipin is identified when the level of expression of a perilipin isoform or mRNA encoding the perilipin isoform is greater in the first population of cells than in the second population of cells. An antagonist of
15 perilipin is identified when the level of expression of a perilipin isoform or mRNA encoding the perilipin isoform is less in the first population of cells than in the second population of cells.

The present invention also provides methods of screening for an agent that modulates the activity of a perilipin isoform, comprising: (a) contacting a first population of
20 cells expressing perilipin isoform with a candidate agent; (b) contacting a second population of cells expressing said perilipin isoform with a control agent; and (c) comparing the level of phosphorylation of said perilipin isoform or the level of induction of a cellular second messenger in the first and second populations of cells. An agonist of perilipin is identified when the level of phosphorylation is greater or the level of induction of the cellular second
25 messenger is greater in the first population of cells than in the second population of cells. An antagonist of perilipin is identified when the level of phosphorylation is less or the level of induction of the cellular second messenger is less in the first population of cells than in the second population of cells.

The present invention also provides methods of identifying an agent to be tested for
30 an ability to modulate body weight, body fat, muscle mass, lipid metabolism, the onset, development or progression of a lipid metabolic disorder (*e.g.*, lipodystrophies), the onset, development or progression of a body weight disorder (*e.g.*, obesity), the onset, development or progression of a disorder characterized by lipid accumulation (*e.g.*, atherosclerosis) or the onset, development or progression of diabetes, comprising: (a)
35 contacting a perilipin isoform or a fragment thereof with a candidate agent for a time sufficient to form perilipin isoform or fragment/agent complexes; and (b) measuring

perilipin isoform or fragment/agent complex level, so that if the level measured differs from that measured in the absence of the candidate agent, then an agent to be tested for an ability to modulate body weight, body fat, muscle mass, lipid metabolism, the onset, development or progression of a lipid metabolic disorder (*e.g.*, lipodystrophies), the onset, development or progression of a body weight disorder (*e.g.*, obesity), the onset, development or
5 progression of a disorder characterized by lipid accumulation (*e.g.*, atherosclerosis) or the onset, development or progression of diabetes is identified.

The present invention also provides methods of identifying an agent to be tested for an ability to modulate body weight, body fat, muscle mass, lipid metabolism, the onset, development or progression of a lipid metabolic disorder (*e.g.*, lipodystrophies), the onset,
10 development or progression of a body weight disorder (*e.g.*, obesity), the onset, development or progression of a disorder characterized by lipid accumulation (*e.g.*, atherosclerosis) or the onset, development or progression of diabetes, comprising: (a) contacting a population of cells expressing a perilipin isoform with a candidate agent for a time sufficient to form perilipin isoform/agent complexes; and (b) measuring perilipin
15 isoform/agent complex level, so that if the level measured differs from that measured in the absence of the candidate agent, then an agent to be tested for an ability to modulate body weight, body fat, muscle mass, lipid metabolism, the onset, development or progression of a lipid metabolic disorder (*e.g.*, lipodystrophies), the onset, development or progression of a body weight disorder (*e.g.*, obesity), the onset, development or progression of a disorder
20 characterized by lipid accumulation (*e.g.*, atherosclerosis) or the onset, development or progression of diabetes is identified.

The present invention also provides methods of identifying an agent to be tested for an ability to modulate body weight, body fat, muscle mass, lipid metabolism, the onset, development or progression of a lipid metabolic disorder (*e.g.*, lipodystrophies), the onset,
25 development or progression of a body weight disorder (*e.g.*, obesity), the onset, development or progression of a disorder characterized by lipid accumulation (*e.g.*, atherosclerosis) or the onset, development or progression of diabetes, comprising: (a) contacting a population of cells expressing a perilipin isoform with a candidate agent; and (b) measuring the level of phosphorylation of the perilipin isoform, the level of induction of
30 a cellular second messenger, or the level of triacylglycerol, nonesterified fatty acids or β -hydroxybutyrate, so that if the level measured differs from that measured in the absence of the candidate agent, then a compound to be tested for an ability to modulate body weight, body fat, muscle mass, lipid metabolism, the onset, development or progression of a lipid metabolic disorder (*e.g.*, lipodystrophies), the onset, development or progression of a body
35 weight disorder (*e.g.*, obesity), the onset, development or progression of a disorder

characterized by lipid accumulation (*e.g.*, atherosclerosis) or the onset, development or progression of diabetes is identified.

The present invention also provides method of screening for or identifying an agent that modulates the expression of a perilipin isoform, comprising: (a) administering a candidate agent to a first animal or group of animals; (b) administering a control agent to a
5 second animal or group of animals; and (c) comparing the level of expression of the perilipin isoform or of mRNA encoding the perilipin isoform in the first and second groups. An agonist of perilipin is identified when the level of expression is greater in the first group than in the second group. An antagonist of perilipin is identified when the level of expression is less in the first group than in the second group.

10 The present invention also provides methods of screening for or identifying an agent that modulates one or more activities of a perilipin isoform, comprising: (a) administering a candidate agent to a first animal or group of animals; (b) administering a control agent to a second animal or group of animals; and (c) comparing the level of induction of a cellular
15 second messenger, the level of phosphorylation of the perilipin isoform, or the level of triacylglycerol, nonesterified fatty acids or β -hydroxybutyrate in the first and second groups. Such activities can be assessed by techniques well-known in the art or described herein. An agonist of a perilipin isoform is identified when the level of induction of the cellular second messenger, level of phosphorylation of the perilipin isoform, or the level of triacylglycerol, nonesterified fatty acids or β -hydroxybutyrate is greater in the first group than in the second
20 group. An antagonist of a perilipin isoform is identified when the level of induction of the cellular second messenger, level of phosphorylation of the perilipin isoform, or the level of triacylglycerol, nonesterified fatty acids or β -hydroxybutyrate is less in the first group than in the second group.

The present invention also provides methods for identifying an agent that modulates
25 the body weight, body fat or muscle mass in an animal, comprising: (a) administering to an animal or group of animals a candidate agent that binds to one or more perilipin isoforms, modulates the expression of one or more perilipin isoforms or modulates one or more activities of one or more perilipin isoforms; and (b) determining whether the candidate agent modulates the body weight, body fat or muscle mass in the animal or animals relative
30 to an untreated control animal or animals, so that if the candidate agent modulates the body weight or muscle mass, then an agent that modulates the body weight, body fat or muscle mass of an animal is identified. Body weight, body fat or muscle mass that can be assessed using techniques well-known to one of skill in the art or described herein.

The present invention also provides methods for identifying an agent that modulates
35 the onset, development or progression of diabetes in an animal, comprising: (a) administering to an animal or group of animals having or predisposed to diabetes a

candidate agent that binds to one or more perilipin isoforms, modulates the expression of one or more perilipin isoforms or modulates one or more activities of one or more perilipin isoforms; and (b) determining whether the candidate agent modulates blood glucose levels, insulin sensitivity, or one or more signs or symptoms of diabetes in the animal or animals relative to an untreated animal or animals, so that if the candidate agent modulates blood
5 glucose levels, insulin sensitivity or one or more signs or symptoms of diabetes, then an agent that modulates the onset, development or progression of diabetes in an animal is identified.

The present invention also provides methods for identifying an agent that modulates lipid metabolism in an animal, comprising: (a) administering to an animal or group of
10 animals a candidate agent that binds to one or more perilipin isoforms, modulates the expression of one or more perilipin isoforms or modulates one or more activities of one or more perilipin isoforms; and (b) determining whether the candidate agent modulates lipid metabolism in the animal or animals relative to an untreated control animal or animals, so that if the candidate agent modulates the lipid metabolism, then an agent that modulates
15 lipid metabolism in an animal is identified. Lipid metabolism can be assessed by techniques well-known in the art or described herein.

The present invention further provides pharmaceutical compositions that modulate the activity, expression or/and phosphorylated state of perilipin. In particular, the pharmaceutical compositions may be agonists or antagonists of perilipin. Antagonists may
20 act by competitively inhibiting another perilipin agonist or antagonist, by blocking the interaction of activated perilipin with its downstream signaling pathway, by inhibiting transcription of the perilipin gene, by inhibiting processing or translation of the perilipin mRNA, or by inhibiting post-translational processing of perilipin. Agonists may act by activating and/or enhancing the natural biological effects of the perilipin signal transduction
25 pathway or its expression.

In another aspect, the present invention provides methods and compositions for preventing and/or treating diseases and disorders characterized by aberrant perilipin expression and/or activity in an animal. The present invention provides methods of preventing and/or treating body weight disorders in animals, preferably in companion
30 animals, livestock and poultry, and more preferably in humans, said methods comprising administering pharmaceutical formulations which modulate perilipin expression and/or activity. In particular, pharmaceutical compositions that enhance body weight and performance, or that reduce body weight and ameliorate signs or symptoms associated with obesity, may be administered to humans. In addition, pharmaceutical compositions that
35 enhance body weight and performance, or that reduce body weight and ameliorate obesity, may be administered to livestock or poultry.

The present invention also provides methods of preventing and/or treating diabetes in animals, preferably in companion animals, livestock and poultry, and more preferably in humans, said methods comprising administering pharmaceutical formulations which modulate perilipin expression and/or activity. In particular, pharmaceutical compositions that delay or prevent the onset, development or progression of diabetes (*e.g.*, diabetes
5 associated and unassociated with obesity) may be administered to animals, preferably companion animals, livestock and poultry, and more preferably humans.

The present invention also provides methods of preventing and/or treating lipid metabolic disorders (*i.e.*, disorders characterized by inappropriate lipid metabolism) and disorders characterized by lipid accumulation in animals, preferably in companion animals,
10 livestock and poultry, and more preferably in humans, said methods comprising administering pharmaceutical formulations which modulate perilipin expression and/or activity. In particular, pharmaceutical compositions that delay or prevent the onset, development or progression of such disorders may be administered to animals, preferably companion animals, livestock and poultry, and more preferably humans.

15 In another aspect, the present invention provides methods and compositions for detecting, diagnosing, or monitoring the development or progression of diseases or disorders characterized by aberrant perilipin expression and/or activity such as, *e.g.*, lipid metabolic disorders, weight disorders (*e.g.*, obesity), and diabetes. In yet another aspect, the present invention provides kits comprising one or more agents identified in the screening
20 assays of the invention, and instructions for use.

3.1. Definitions

The terms "Lep^{ob/ob}" and "ob/ob" are used herein interchangeably to refer to leptin deficient mice.

25 The terms "Lepr^{db/db}" and "db/db" are used herein interchangeably to refer to leptin resistant mice.

The term "aberrant" as used herein in the context of perilipin expression means that the expression level of one or more perilipin isoforms is increased or decreased in cells, tissues, or a subject compared with the expression level in cells or tissues obtained from a
30 normal subject or a subject free from a lipid metabolic disorder, weight disorder or diabetes, or a reference level. The expression level of one or more perilipin isoforms can be determined by methods described herein or known to those of ordinary skill in the art.

The term "aberrant" as used herein in the context of perilipin activity means that the activity level of one or more perilipin isoforms is increased or decreased in cells or tissues
35 obtained from a subject compared with the activity level in cells or tissues obtained from a normal subject or a subject free from a lipid metabolic disorder, weight disorder or diabetes,

or a reference level. The activity level of one or more perilipin isoforms can be determined by methods described herein or known to those of ordinary skill in the art.

The term "analog" as used herein in the context of polypeptides refers to a first polypeptide that possesses a similar or identical function as a second polypeptide (*e.g.*, a perilipin isoform or anti-perilipin antibody) but does not necessarily comprise a similar or identical amino acid sequence of the second protein, or possess a similar or identical structure as the second protein. A polypeptide that has a similar amino acid sequence refers to a polypeptide that satisfies at least one of the following: (a) a first polypeptide having an amino acid sequence that is at least 30%, at least 35%, at least 40%, at least 45%, at least 50%, at least 55%, at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95% or at least 99% identical to the amino acid sequence of a second polypeptide (*e.g.*, a perilipin isoform or anti-perilipin antibody); (b) a first polypeptide encoded by a nucleotide sequence that hybridizes under stringent conditions to a nucleotide sequence encoding a second polypeptide (*e.g.*, a perilipin isoform or anti-perilipin antibody) of at least 5 contiguous amino acid residues, at least 10 contiguous amino acid residues, at least 15 contiguous amino acid residues, at least 20 contiguous amino acid residues, at least 25 contiguous amino acid residues, at least 40 contiguous amino acid residues, at least 50 contiguous amino acid residues, at least 60 contiguous amino residues, at least 70 contiguous amino acid residues, at least 80 contiguous amino acid residues, at least 90 contiguous amino acid residues, at least 100 contiguous amino acid residues, at least 125 contiguous amino acid residues, or at least 150 contiguous amino acid residues; and (c) a first polypeptide encoded by a nucleotide sequence that is at least 30%, at least 35%, at least 40%, at least 45%, at least 50%, at least 55%, at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95% or at least 99% identical to the nucleotide sequence encoding a second polypeptide (*e.g.*, a perilipin isoform or anti-perilipin antibody). A first polypeptide with similar structure to a second polypeptide refers to a first polypeptide that has a similar secondary, tertiary or quaternary structure to a second polypeptide. The structure of a polypeptide can be determined by methods known to those skilled in the art, including but not limited to, peptide sequencing, X-ray crystallography, nuclear magnetic resonance, circular dichroism, and crystallographic electron microscopy.

To determine the percent identity of two amino acid sequences or of two nucleic acids, the sequences are aligned for optimal comparison purposes (*e.g.*, gaps can be introduced in the sequence of a first amino acid or nucleic acid sequence for optimal alignment with a second amino or nucleic acid sequence). The amino acid residues or nucleotides at corresponding amino acid positions or nucleotide positions are then compared. When a position in the first sequence is occupied by the same amino acid

residue or nucleotide as the corresponding position in the second sequence, then the molecules are identical at that position. The percent identity between the two sequences is a function of the number of identical positions shared by the sequences (% identity = # of identical positions/total # of positions (e.g., overlapping positions) x 100). In one embodiment, the two sequences are the same length.

5 The determination of percent identity between two sequences can be accomplished using a mathematical algorithm. A preferred, non-limiting example of a mathematical algorithm utilized for the comparison of two sequences is the algorithm of Karlin and Altschul (1990) *Proc. Natl. Acad. Sci. USA* 87:2264-2268, modified as in Karlin and Altschul (1993) *Proc. Natl. Acad. Sci. USA* 90:5873-5877. Such an algorithm is
 10 incorporated into the NBLAST and XBLAST programs of Altschul, et al. (1990) *J. Mol. Biol.* 215:403-410. BLAST nucleotide searches can be performed with the NBLAST program, score = 100, wordlength = 12 to obtain nucleotide sequences homologous to a nucleic acid molecules of the invention. BLAST protein searches can be performed with the XBLAST program, score = 50, wordlength = 3 to obtain amino acid sequences
 15 homologous to a protein molecules of the invention. To obtain gapped alignments for comparison purposes, Gapped BLAST can be utilized as described in Altschul et al. (1997) *Nucleic Acids Res.* 25:3389-3402. Alternatively, PSI-Blast can be used to perform an iterated search which detects distant relationships between molecules (*Id.*). When utilizing BLAST, Gapped BLAST, and PSI-Blast programs, the default parameters of the respective
 20 programs (e.g., XBLAST and NBLAST) can be used. See <http://www.ncbi.nlm.nih.gov>.

Another preferred, non-limiting example of a mathematical algorithm utilized for the comparison of sequences is the algorithm of Myers and Miller, CABIOS (1989). Such an algorithm is incorporated into the ALIGN program (version 2.0) which is part of the CGC sequence alignment software package. When utilizing the ALIGN program for comparing
 25 amino acid sequences, a PAM120 weight residue table, a gap length penalty of 12, and a gap penalty of 4 can be used. Additional algorithms for sequence analysis are known in the art and include ADVANCE and ADAM as described in Torellis and Robotti (1994) *Comput. Appl. Biosci.*, 10:3-5; and FASTA described in Pearson and Lipman (1988) *Proc. Natl. Acad. Sci.* 85:2444-8. Within FASTA, ktup is a control option that sets the sensitivity
 30 and speed of the search. If ktup=2, similar regions in the two sequences being compared are found by looking at pairs of aligned residues; if ktup=1, single aligned amino acids are examined. ktup can be set to 2 or 1 for protein sequences, or from 1 to 6 for DNA sequences. The default if ktup is not specified is 2 for proteins and 6 for DNA. For a further description of FASTA parameters, see
 35 <http://bioweb.pasteur.fr/docs/man/man/fasta.1.html#sect2>, the contents of which are incorporated herein by reference.

The percent identity between two sequences can be determined using techniques similar to those described above, with or without allowing gaps. In calculating percent identity, typically exact matches are counted.

The term "derivative" as used herein in the context of polypeptides refers to a first polypeptide that comprises an amino acid sequence of a second polypeptide (*e.g.*, a perilipin isoform or anti-perilipin antibody), which has been altered by the introduction of amino acid residue substitutions, deletions or additions, or by the covalent attachment of any type of molecule to the second polypeptide. For example, but not by way of limitation, a polypeptide may be modified, *e.g.*, by proteolytic cleavage, linkage to a cellular ligand or other protein, etc. A derivative of a polypeptide may be modified by chemical modifications using techniques known to those of skill in the art (*e.g.*, by acylation, phosphorylation, carboxylation, glycosylation, selenium modification and sulfation). Further, a derivative of a polypeptide may contain one or more non-classical amino acids. A polypeptide derivative may or may not possess a similar or identical function as the polypeptide from which it was derived. In certain embodiments, a derivative of a perilipin isoform retains at least one function of the perilipin isoform from which it was derived. In certain other embodiments, a derivative of a perilipin isoform does not retain any function of the perilipin isoform from which it was derived.

The term "fragment" as used herein refers to a peptide or polypeptide comprising an amino acid sequence of at least 5 contiguous amino acid residues, preferably, at least 10, at least 15, at least 20, at least 25, at least 40, at least 50, at least 60, at least 70, at least 80, at least 90, at least 100, at least 125, at least 150, at least 175, at least 200, at least 250, at least 275, at least 300, at least 325, at least 350, at least 375, at least 400 or more contiguous amino acid residues of the amino acid sequence of another peptide or polypeptide. A fragment of a polypeptide may or may not possess a functional activity of the polypeptide. In certain embodiments, a fragment of a perilipin isoform retains at least one function of the perilipin isoform. In certain other embodiments, a fragment of a perilipin isoform does not retain any function of the perilipin isoform from which it was derived.

The term "functional fragment" as used herein refers to a fragment of peptide or polypeptide comprising an amino acid sequence of at least 5 contiguous amino acid residues, preferably, at least 10, at least 15, at least 20, at least 25, at least 40, at least 50, at least 60, at least 70, at least 80, at least 90, at least 100, at least 125, at least 150, at least 175, at least 200, at least 250, at least 275, at least 300, at least 325, at least 350, at least 375, at least 400 or more contiguous amino acid residues of the amino acid sequence of a another peptide or polypeptide, which retains at least one function or activity of the other peptide or polypeptide. For example, a functional fragment of a perilipin isoform may

retain the ability to bind to an anti-perilipin antibody, the ability to bind to lipid droplets, the ability to interact with PKA, or the ability to interact with protein phosphatase 1.

The term "fusion protein" as used herein refers to a polypeptide that comprises (i) an amino acid sequence of first polypeptide (*e.g.*, a perilipin isoform or fragment thereof) and (ii) an amino acid sequence of a second, heterologous polypeptide. A "perilipin fusion protein" as used herein refers to a polypeptide that comprises (i) an amino acid sequence of a perilipin isoform or a fragment thereof and (ii) an amino acid sequence of a heterologous polypeptide (*i.e.*, a non-perilipin polypeptide or fragment thereof). In one embodiment, a perilipin fusion protein comprises a perilipin isoform or a fragment thereof and a domain such as glutathione-S-transferase. In another embodiment, a perilipin fusion protein comprises a perilipin isoform or fragment thereof and a fragment of an antibody, preferably the Fc domain of an antibody. Perilipin fusion proteins can be made using techniques well-known to one of skill in the art. Fusion proteins can be produced by standard recombinant DNA techniques.

The term "hybridizes under conditions" as used herein describe conditions for hybridization and washing under which nucleotide sequences at least 60% (65%, 70%, preferably 75%) identical to each other typically remain hybridized to each other. Such stringent conditions are known to those skilled in the art and can be found in *Current Protocols in Molecular Biology*, John Wiley & Sons, N.Y. (1989), 6.3.1-6.3.6. In one, non-limiting example stringent hybridization conditions are hybridization at 6X sodium chloride/sodium citrate (SSC) at about 45° C, followed by one or more washes in 0.1XSSC, 0.2% SDS at about 68° C. A preferred, non-limiting example stringent hybridization conditions are hybridization in 6XSSC at about 45° C, followed by one or more washes in 0.2 X SSC, 0.1% SDS at 50-65° C (*i.e.*, one or more washes at 50° C, 55° C, 60° C or 65° C). It is understood that the nucleic acids of the invention do not include nucleic acid molecules that hybridize under these conditions solely to a nucleotide sequence consisting of only A or T nucleotides. In a specific embodiment, a polypeptide hybridizes over its full length to a perilipin isoform, and preferably said polypeptide has at least one function or activity of the perilipin isoform.

The term "isoform" as used herein refers to variants of perilipin that are encoded by the same gene, but that differ in their amino acid composition. There are three known isoforms of perilipin: perilipin A, perilipin B and perilipin C.

"Isolated" or "purified" when used herein to describe a nucleic acid molecule or nucleotide sequence, refers to a nucleic acid molecule or nucleotide sequence which is separated from other nucleic acid molecules which are present in the natural source of the nucleic acid molecule. Preferably, an "isolated" nucleic acid molecule is free of sequences (preferably protein encoding sequences) which naturally flank the nucleic acid (*i.e.*,

sequences located at the 5' and 3' ends of the nucleic acid) in the genomic DNA of the organism from which the nucleic acid is derived. Moreover, an "isolated" nucleic acid molecule, such as a cDNA molecule, can be substantially free of other cellular material, or culture medium when produced by recombinant techniques, or substantially free of chemical precursors or other chemicals when chemically synthesized.

5 "Isolated" or "purified" when used herein to describe a protein or biologically active portion thereof (*i.e.*, a polypeptide, peptide or amino acid fragment), refers to a protein or biologically active portion thereof substantially free of cellular material or other contaminating proteins from the cell or tissue source from which the protein is derived, or substantially free of chemical precursors or other chemicals when chemically synthesized.

10 A protein or biologically active portion thereof (*i.e.*, a polypeptide, peptide or amino acid fragment) that is substantially free of cellular material includes preparations of protein having less than about 30%, 20%, 10%, or 5% (by dry weight) of heterologous protein (also referred to herein as a "contaminating protein").

The term "modulate" when used herein in reference to expression or activity of
15 perilipin refers to any change, *e.g.*, upregulation or downregulation, of the expression or activity of perilipin. Based on the present disclosure, such modulation can be determined by assays known to those of skill in the art or described herein.

The terms "nucleic acids" and "nucleotide sequences" as used herein include DNA molecules (*e.g.*, cDNA or genomic DNA), RNA molecules (*e.g.*, mRNA), combinations of
20 DNA and RNA molecules or hybrid DNA/RNA molecules, and analogs of DNA or RNA molecules. Such analogs can be generated using, for example, nucleotide analogs, which include, but are not limited to, inosine or tritylated bases. Such analogs can also comprise DNA or RNA molecules comprising modified backbones that lend beneficial attributes to the molecules such as, for example, nuclease resistance or an increased ability to cross
25 cellular membranes. The nucleic acids or nucleotide sequences can be single-stranded, double-stranded, may contain both single-stranded and double-stranded portions, and may contain triple-stranded portions, but preferably is double-stranded DNA. In one embodiment, the nucleotide sequences comprise a contiguous open reading frame encoding a perilipin isoform or fragment thereof, *e.g.*, a cDNA molecule.

30 The terms "prevent", "preventing" and "prevention" as used herein refer to the prevention of the onset or recurrence of one or more signs or symptoms associated with a weight disorder (*e.g.*, obesity), diabetes (type 1 or type 2), or a disorder characterized by lipid accumulation (*e.g.*, atherosclerosis), or a lipid metabolic disorder (*e.g.*, a lipodystrophy).

35 The term "prophylactically effective amount" as used herein refers to the amount of an agent that modulates the expression and/or activity of one or more perilipin isoforms, or

the amount of a composition comprising an agent that modulates the expression and/or activity of one or more perilipin isoforms sufficient to prevent the onset or recurrence of one or more signs or symptoms associated with a weight disorder (*e.g.*, obesity), diabetes (type 1 or type 2), a metabolic disorder (*e.g.*, a lipodystrophy) or a disorder characterized by lipid accumulation (*e.g.*, atherosclerosis).

5 The terms “treat”, “treating”, and “treatment” as used herein refer: to the reduction in the severity of one or more signs or symptoms associated with a weight disorder (*e.g.*, obesity), diabetes (type 1 or type 2), a lipid metabolic disorder (*e.g.*, a lipodystrophy) or a disorder characterized by lipid accumulation (*e.g.*, atherosclerosis); the reduction in insulin resistance in an animal; the improved secretion of insulin in an animal; the reduction in the
10 body weight of an animal with a weight disorder characterized by increased weight gain (*e.g.*, obesity); the reduction in body fat of an animal; the increase in the body weight of an animal with a weight disorder characterized by low body weight (*e.g.*, anorexia or cachexia); the increase in muscle mass in an animal; the increase in lipid metabolism in an animal; the reduction of glucose intolerance in an animal.

15 The term “therapeutically effective amount” as used herein refers to the amount of an agent that modulates the expression and/or activity of one or more perilipin isoforms, or the amount of a composition comprising an agent that modulates the expression and/or activity of one or more perilipin isoforms sufficient to: reduce the severity of one or more signs or symptoms associated with a weight disorder (*e.g.*, obesity), diabetes (type 1 or type
20 2), a lipid metabolic disorder (*e.g.*, a lipodystrophy) or a disorder characterized by lipid accumulation (*e.g.*, atherosclerosis); reduce in insulin resistance in an animal; improve the secretion of insulin in an animal; reduce in the body weight of an animal with a body weight disorder characterized by weight gain (*e.g.*, obesity); reduce body fat in an animal; increase in the body weight of an animal with a weight disorder characterized by low body weight
25 (*e.g.*, anorexia or cachexia); increase muscle mass in an animal; increase lipid metabolism in an animal; reduce glucose intolerance in an animal.

4. DESCRIPTION OF THE DRAWINGS

FIGS. 1A-1C. Generation of *plin*^{-/-} mice. **A.** Targeting strategy. Exons 1-6 are
30 represented by filled boxes. A replacement vector that replaces parts of exon 2 and intron 2 between a *Bst*XI and an *Apa*I site with an IRES- β -galactosidase and the *neo*^r gene. Interruption of exon 2 occurs at codon 183 at a *Bst*XI site. A thymidine kinase cassette (TK) was attached to the 3'-end of the targeting vector. H, *Hind*III; Xb, *Xba*I; Ap, *Apa*I; BX, *Bst*XI, TK, thymidine kinase. **B.** Southern blotting of tail DNA following *Xba*I
35 digestion. Use of a 3' DNA probe (see A above) detects an 8.5-kb band encompassing exons 2-6 in wild-type DNA and a 7.0-kb band encompassing part of the *neo* gene and the

exons 3-6 in *plin*^{-/-} DNA. **C.** Western blotting of cell extracts from epididymal and subcutaneous fat (*left*) and testes (*right*). In adipose tissues, perilipins A and B are detected, and in testes, perilipins A and C are detected in wild-type (Londos et al., 1999, Semin. Cell Dev. Biol. 19:51-58). All these isoforms are products of the *plin* gene, and are undetectable in *plin*^{-/-} samples. In wild-type mice, perilipin expression is much higher in adipose tissue
5 than in testes.

FIGS. 2A-2F. Phenotypic effect of perilipin inactivation on body weight, adipose depots, lipid contents and muscle mass in *plin*^{-/-} mice. **A.** Body weights of male *plin*^{+/+} (n=11), *plin*^{+/-} (n=15) and *plin*^{-/-} (n=8) mice. **B.** Body weights of female *plin*^{+/+} (n=6), *plin*^{+/-} (n=24) and *plin*^{-/-} (n=12) mice. **C.** Mass of adipose depots in *plin*^{-/-} and *plin*^{+/+} mice. **D.** Total carcass lipid content of *plin*^{+/+} and *plin*^{-/-} mice. **E.** Total carcass triacylglycerol content of *plin*^{+/+} and *plin*^{-/-} mice. **F.** Total carcass protein content of *plin*^{+/+} and *plin*^{-/-} mice. **G.** Weight of isolated gastrocnemius muscle in *plin*^{+/+} and *plin*^{-/-} mice as % total body weight
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FIGS. 3A-3D. Effect of perilipin inactivation on adipose tissue. **A.** Histology of adipose tissues. Sections of white adipose tissue (WAT, subcutaneous, epididymal) and brown adipose tissue (BAT, interscapular) depots in *plin*^{+/+} (*left*) and *plin*^{-/-} mice (*right*). Note difference in magnification between WAT and BAT. **B.** Size distribution of fat cells in epididymal fat depot in *plin*^{+/+} and *plin*^{-/-} mice. The area of individual fat cells was determined in sections of epididymal fat. **C.** DNA content of epididymal fat of *plin*^{+/+} and *plin*^{-/-} mice. **D.** Average size of brown adipocytes in interscapular fat depot in *plin*^{+/+} and *plin*^{-/-} mice.
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FIGS. 4A-4E. **A.** Effect on a 48-h fast on body weight and plasma parameters in *plin*^{+/+} and *plin*^{-/-} mice. **B.** Western blotting of HSL extracted from epididymal fat. **C.** HSL activity of isolated adipocytes. HSL activity was determined in total cell lysate of subcutaneous and epididymal fat isolated from *plin*^{+/+} and *plin*^{-/-} mice as described in Methods. The activity is presented as per gram adipose tissue weight. **D.** Rate of lipolysis in isolated adipocytes. Adipocytes were isolated from epididymal fat of *plin*^{+/+} and *plin*^{-/-} mice. Lipolytic activity was measured as described in Methods in the absence and presence of CL 316,243 (CL) (2 μM). **E.** Lipolysis in *plin*^{+/+} and *plin*^{-/-} mice. The lipolysis products, glycerol and nonesterified (free) fatty acids (NEFA) in plasma were measured before and after isoproterenol (isopro) (10 mg/kg IP, *top*) and CL 316,243 (CL) (0.1 mg/kg IP, *bottom*).
30
35 *p<0.05; **p<0.001.

FIGS. 5A-5E. *A.* Body temperature of *plin*^{+/+} and *plin*^{-/-} mice kept at 21 °C. Number of animals studied: male *plin*^{+/+} 16, *plin*^{-/-} 12; female *plin*^{+/+} 6, *plin*^{-/-}, 11. *B.* Body temperature of mice on exposure to an ambient temperature of 4 °C in the absence of food. The fast started at the time the mice were first exposed to this temperature (n=6 for both groups, all male). *C.* Body temperature of mice on exposure to an ambient temperature of 4 °C in the fed state (n=5 for both groups, all male). * *plin*^{-/-} significantly different from *plin*^{+/+}, p<0.005. *D.* Representative oxygen consumption (VO₂) tracings from indirect calorimetry in *plin*^{+/+} and *plin*^{-/-} mice fed a diet containing 35% fat. VO₂ peaks correspond to periods of activity and baselines represent resting values. *E.* Representative VO₂ tracings in 14-week old *db/db/plin*^{+/+} and *db/db/plin*^{-/-} mice on regular chow.

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FIGS. 6A-6D. *A.* Epididymal fat pad mass of groups of *plin*^{+/+} and *plin*^{-/-} mice on regular chow or after they were fed a 35% fat (HF) diet for 3.5 months (male mice, n=5 per group). The pre-HF diet data were the same as those presented in Fig. 2C. *B.* Total carcass fat content of *plin*^{+/+} and *plin*^{-/-} mice on regular chow or after they were fed a 35% fat (HF) diet for 3.5 months (male mice, n=5 per group). The pre-HF diet data were the same as those presented in Fig. 2d. *C.* Photographs of representative *db/db/plin*^{+/+}, *db/db/plin*^{-/-}, *plin*^{+/+} and *plin*^{-/-} mice. *D.* Magnetic resonance imaging (MRI) of body fat distribution. *Top*, MRI section of *plin*^{+/+} (left panel) *plin*^{-/-} (right panel) mice. *Bottom*, MRI of *db/db/plin*^{+/+} (left panel) and *db/db/plin*^{-/-} (right panel) mice. Each of four *in vivo* MRIs represents a cross section from the abdominal region of representative mice of different genotypes. The spin echo MRIs were selected from multislice sets acquired with a repetition time of 2.5 seconds, an echo time of 12 milliseconds, a 6- by 6-cm field of view, 1.5-mm slices and 128- or 256-phase encode steps. Respiratory gating was applied in order to reduce motion artifacts. The proton NMR spectra presented above each image represents the water and lipid signals from the whole mouse. The spectra were acquired with a single pulse-acquire sequence. Each spectrum was integrated in the regions from 6.5 ppm to 3.5 ppm and from 3.5 ppm to 0.5 ppm to estimate whole-body relative water and lipid concentrations.

FIGS. 7A-7B. *A.* Body weight of male *db/db* (triangle), *plin*^{+/+} (square), and *db/db/plin*^{-/-} (circle) mice over a period of 4 to 17 weeks. *B.* Body of weight of female *db/db* (triangle), *plin*^{+/+} (square), and *db/db/plin*^{-/-} (circle) mice over a period of 4 to 22 weeks.

FIGS. 8A-8B. *A.* Body weight of male *db/db* (square), *plin*^{+/+} (diamond), and *db/db/plin*^{-/-} (triangle) mice over a period of 6 to 27 weeks. *B.* Body of weight of female

db/db (square), *plin*^{+/+} (diamond), and *db/db/plin*^{-/-} (triangle) mice over a period of 6 to 24 weeks.

FIG. 9. Steps in β -oxidation.

5 **FIG. 10.** Expression of fatty acyl-CoA dehydrogenase. The mRNA expression of the β -oxidation enzyme fatty acyl-CoA dehydrogenase, in particular very long chain fatty acyl-CoA dehydrogenase (VLCAD) and long chain fatty acyl-CoA dehydrogenase (LCAD), in white adipose tissue (WAT), brown adipose tissue (BAT), the liver, the heart, and muscle of wild-type (*plin*^{+/+}) mice and *plin*^{-/-} mice as determined by Northern blot analysis.

10 **FIG. 11.** Expression of trifunctional protein. The mRNA expression of thiolase (trifunctional protein β) and LCHAD (trifunctional protein α) in white adipose tissue (WAT), brown adipose tissue (BAT), the liver, the heart, and muscle of wild-type (*plin*^{+/+}) mice and *plin*^{-/-} mice as determined by Northern blot analysis.

15 **FIG. 12.** Expression of uncouple proteins (UCPs). The mRNA expression of UCP-1 and UCP-2 in white adipose tissue (WAT), brown adipose tissue (BAT), the liver, the heart, and muscle of wild-type (*plin*^{+/+}) mice and *plin*^{-/-} mice as determined by Northern blot analysis.

20 **FIGS. 13A-13B.** **A.** Fasting plasma glucose levels. The concentration of glucose in the plasma of *Lep*^{+/+}/*plin*^{+/+} mice, *Lep*^{+/+}/*plin*^{-/-} mice, *Lep*^{ob/ob}/*plin*^{+/+} mice, and *Lep*^{ob/ob}/*plin*^{-/-} mice after a 10 hour fast. **B.** Fasting plasma insulin levels. The concentration of insulin in the plasma of *Lep*^{+/+}/*plin*^{+/+} mice, *Lep*^{+/+}/*plin*^{-/-} mice, *Lep*^{ob/ob}/*plin*^{+/+} mice, and
25 *Lep*^{ob/ob}/*plin*^{-/-} mice after a 10 hour fast.

FIGS. 14A-14B. Glucose Intolerance Test. *Lep*^{+/+}/*plin*^{+/+} mice, *Lep*^{+/+}/*plin*^{-/-} mice, *Lep*^{ob/ob}/*plin*^{+/+} mice, and *Lep*^{ob/ob}/*plin*^{-/-} mice were fasted for 10 hours prior to the administration of glucose for the glucose intolerance test. **A.** The concentration of plasma
30 glucose at 0, 30, 60, and 90 minutes after the administration of 1.5 g/kg glucose i.p. to *Lep*^{+/+}/*plin*^{+/+} mice (diamond), *Lep*^{+/+}/*plin*^{-/-} mice (square), *Lep*^{ob/ob}/*plin*^{+/+} mice (triangle), and *Lep*^{ob/ob}/*plin*^{-/-} mice (circle). **B.** The concentration of plasma insulin at 0, 30, 60, and 90 minutes after the administration of 1.5 g/kg glucose i.p. to *Lep*^{+/+}/*plin*^{+/+} mice (diamond), *Lep*^{+/+}/*plin*^{-/-} mice (square), *Lep*^{ob/ob}/*plin*^{+/+} mice (triangle), and *Lep*^{ob/ob}/*plin*^{-/-} mice (circle).

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5. DETAILED DESCRIPTION OF THE INVENTION

The present invention encompasses screening assays to identify agents that modulate the activity and/or expression of perilipin, as a means of identifying agents that would be useful for the prevention and/or treatment of disorders related to body weight and/or inappropriate regulation of lipid metabolism. In particular, the present invention provides *in vitro* and *in vivo* assays to identify compounds that modulate the expression and/or activity of one or more perilipin isoforms. The present invention also provides *in vitro* and *in vivo* assays to identify compounds that modulate the expression and/or activity of a signal transduction molecule such as an enzyme (*e.g.*, a phosphatase, kinase or phosphodiesterase) that modulates the expression and/or activity of perilipin, and thereby affects the expression and/or activity of perilipin.

The invention also encompasses pharmaceutical compositions comprising compounds which modulate the activity and/or expression of the perilipin protein for the prevention and/or treatment of disorders related to body weight and/or inappropriate regulation of lipid metabolism. In particular, the pharmaceutical compositions may be agonists or antagonists of perilipin. Antagonists may act by competitively inhibiting another perilipin agonist or antagonist, by blocking the interaction of activated perilipin with its downstream signaling pathway, by inhibiting transcription of the perilipin gene, by inhibiting processing or translation of the perilipin mRNA, or by inhibiting the post-translational processing of one or more perilipin isoforms. Agonists may act by activating and/or enhancing the natural biological effects of the perilipin signal transduction pathway or its expression. In one embodiment, an agonist increases the phosphorylation of one or more perilipin isoforms.

The present invention provides methods of preventing and/or treating body weight disorders in animals, preferably in companion animals, livestock and poultry and more preferably humans, comprising administering pharmaceutical compositions which modulate perilipin expression and/or activity. In one embodiment, pharmaceutical compositions comprising one or more compounds that enhance body weight and performance of an animal by modulating perilipin expression and/or activity are administered to an animal in an effective amount. In another embodiment, pharmaceutical compositions comprising one or more compounds that reduce body weight and ameliorate symptoms associated with obesity by modulating perilipin expression and/or activity are administered to animal in need thereof in an effective amount. In another embodiment, compounds that enhance lipid metabolism and increase muscle mass in an animal by modulating perilipin expression and/or activity are administered to an animal in need thereof in an effective amount. In another embodiment, pharmaceutical compositions comprising one or more compounds that enhance lipid accumulation in an animal by modulating perilipin expression and/or activity

are administered to an animal in need thereof in an effective amount. In yet another embodiment, pharmaceutical compositions comprising one or more compounds that ameliorate or delay the onset or progression of diabetes by modulating perilipin expression and/or activity are administered to an animal in need thereof in an effective amount.

The present invention also provides methods of detecting, diagnosing, or monitoring
5 the development or progression of diseases and disorders characterized by aberrant expression and/or activity of one or more perilipin isoforms such as lipid metabolic disorders, weight disorders, and diabetes. In particular, the present invention provides methods of diagnosing or detecting a predisposition for obesity in an animal by detecting the level of expression and/or activity of perilipin.

10

5.1. Screening Assays to Identify Compounds Which Modulate Perilipin Activity and/or Expression

The invention provides methods for identifying agents (*e.g.*, candidate compounds or test compounds) that bind to perilipin or have a stimulatory or inhibitory effect on the expression and/or activity of perilipin. In a specific embodiment, the invention provides
15 methods for identifying agents that modulate the phosphorylation of one or more perilipin isoforms and thereby affect the activity of one or more perilipin isoforms. The present invention also provides methods of identifying agents that modulate the expression and/or activity of an enzyme, such as a phosphatase or kinase, involved in the regulation of the phosphorylated state of one or more perilipin isoforms. Examples of agents, compounds,
20 candidate compounds, or test compounds include, but are not limited to, nucleic acids (*e.g.*, DNA and RNA), carbohydrates, lipids, proteins, peptides (including cyclic peptides), peptidomimetics, antibodies, antibody fragments, small molecules and other drugs. Small molecules include, but are not limited to, peptides, peptidomimetics, amino acids, amino acid analogs, polynucleotides, polynucleotide analogs, nucleotides, nucleotide analogs,
25 organic or inorganic compounds (*i.e.*, including heteroorganic and organometallic compounds) having a molecular weight less than about 10,000 grams per mole, organic or inorganic compounds having a molecular weight less than about 5,000 grams per mole, organic or inorganic compounds having a molecular weight less than about 1,000 grams per mole, organic or inorganic compounds having a molecular weight less than about 500 grams
30 per mole, and salts, esters, and other pharmaceutically acceptable forms of such compounds. In a specific embodiment, the agent is a small molecule.

Compounds that can be tested and identified in the methods described herein can include, but are not limited to, compounds obtained from any commercial source, including
Aldrich (1001 West St. Paul Ave., Milwaukee, WI 53233), Sigma Chemical (P.O. Box
35 14508, St. Louis, MO 63178), Fluka Chemie AG (Industriestrasse 25, CH-9471 Buchs, Switzerland), Fluka Chemical Co. (980 South 2nd Street, Ronkonkoma, NY 11779),

Eastman Chemical Company, Fine Chemicals (P.O. Box 431, Kingsport, TN 37662), and Boehringer Mannheim GmbH (Sandhofer Strasse 116, D-68298 Mannheim). Any kind of natural products may be screened using the methods of the invention, including microbial, fungal, plant and animal extracts.

The compounds that can be tested for their ability to modulate perilipin expression
5 and/or activity include, but are not limited to, agents that modulate the phosphorylation of perilipin, such as agents that modulate PKA activity and agents that modulate protein phosphatase 1 (PP1) activity. Examples of such agents include, but are not limited to, β -adrenegic agonist (*e.g.*, isoproterenol) and phosphodiesterase inhibitors (*e.g.*, theophylline, isobutylmethylxanthine, and papaverine). In certain embodiments, the compounds to be
10 tested do not include agents that modulate PKA activity, agents that modulate protein phosphatase 1 (PP1) activity, or agents that modulate phosphodiesterase activity. In certain other embodiments, the agents tested for their ability to modulate perilipin expression and/or activity do not include isoproterenol, theophylline, isobutylmethylxanthine, or papaverine. In certain other embodiments, the agents tested for their ability to modulate
15 perilipin expression and/or activity do not include a compound which was known or previously used in the prevention, treatment or amelioration of one or more signs or symptoms associated with a weight disorder, a disorder characterized by inappropriate lipid metabolism, a disorder characterized by lipid accumulation, or diabetes.

Further, diversity libraries of agents, including small molecules, may be utilized.
20 Such libraries may be, *e.g.*, commercially obtained from Specs and BioSpecs B.V. (Rijswijk, The Netherlands), Chembridge Corporation (San Diego, CA), Contract Service Company (Dolgoprudgy, Moscow Region, Russia), Comgenex USA Inc. (Princeton, NJ), Maybridge Chemicals Ltd. (Cornwall PL24 OHW, United Kingdom), and Asinex (Moscow, Russia).

25 Still further, agents can be obtained using any of the numerous approaches in combinatorial library methods known in the art, including: biological libraries; spatially addressable parallel solid phase or solution phase libraries; synthetic library methods requiring deconvolution; the "one-bead one-compound" library method; and synthetic library methods using affinity chromatography selection. The biological library approach is
30 limited to peptide libraries, while the other four approaches are applicable to peptide, non-peptide oligomer or small molecule libraries of compounds (Lam, 1997, Anticancer Drug Des. 12:145; U.S. Patent No. 5,738,996; and U.S. Patent No. 5,807,683, each of which is incorporated herein in its entirety by reference).

Examples of methods for the synthesis of molecular libraries can be found in the art,
35 for example in: DeWitt et al., 1993, Proc. Natl. Acad. Sci. USA 90:6909; Erb et al., 1994, Proc. Natl. Acad. Sci. USA 91:11422; Zuckermann et al., 1994, J. Med. Chem. 37:2678;

Cho et al., 1993, Science 261:1303; Carrell et al., 1994, Angew. Chem. Int. Ed. Engl. 33:2059; Carrell et al., 1994, Angew. Chem. Int. Ed. Engl. 33:2061; and Gallop et al., 1994, J. Med. Chem. 37:1233, each of which is incorporated herein in its entirety by reference.

Libraries of compounds may be presented, *e.g.*, presented in solution (*e.g.*, Houghten, 1992, Bio/Techniques 13:412-421), or on beads (Lam, 1991, Nature 354:82-84),
5 chips (Fodor, 1993, Nature 364:555-556), bacteria (U.S. Patent No. 5,223,409), spores (Patent Nos. 5,571,698; 5,403,484; and 5,223,409), plasmids (Cull et al., 1992, Proc. Natl. Acad. Sci. USA 89:1865-1869) or phage (Scott and Smith, 1990, Science 249:386-390; Devlin, 1990, Science 249:404-406; Cwirla et al., 1990, Proc. Natl. Acad. Sci. USA 87:6378-6382; and Felici, 1991, J. Mol. Biol. 222:301-310), each of which is incorporated
10 herein in its entirety by reference.

5.1.1. Assays for Agents that Interact with Perilipin

In one embodiment, agents (*i.e.*, candidate compounds) that interact with (*i.e.*, bind to) perilipin (*i.e.*, one or more perilipin isoforms) or a fragment thereof (*e.g.*, a functionally
15 active fragment), or a perilipin fusion protein are identified in a cell-based assay system. In accordance with this embodiment, cells expressing perilipin, a fragment thereof, or a perilipin fusion protein are contacted with a candidate compound or a control compound and the ability of the candidate compound to interact with perilipin, a fragment thereof, or a perilipin fusion protein is determined. If desired, a plurality (*e.g.* a library) of candidate
20 compounds may be screened using this assay.

Cells used in these assays can be, for example, of prokaryotic origin (*e.g.*, *E. coli*) or eukaryotic origin (*e.g.*, yeast or mammalian). The cells can express one or more perilipin isoforms endogenously (*e.g.*, steroidogenic cells and adipocytes) or be genetically engineered to express one or more perilipin isoforms or a fragment thereof, or a perilipin
25 fusion protein. Primary cells or cell lines can be used in the screening assays of the invention. Further, the cells can be obtained from recombinant, transgenic cell lines. For example, cells can be obtained from *db/db* or *ob/ob* mice and transformed into continuous cell lines. Examples of techniques which can be used to derive a continuous cell line from the transgenic animals are known to those of skill in the art, see, *e.g.*, Small et al., 1985,
30 Mol. Cell Biol. 5:642-648.

In certain instances, perilipin (*i.e.*, one or more perilipin isoforms) or a fragment thereof, or a perilipin fusion protein is labeled such that binding of the candidate compound to perilipin, a perilipin fragment, or a perilipin fusion protein can be determined by detecting the labeled compound in a complex. In certain other instances, a candidate
35 compound is labeled such that binding of the candidate compound to perilipin (*i.e.*, one or more perilipin isoforms), a perilipin fragment, or a perilipin fusion protein can be detected

by detecting the labeled candidate compound in a complex. Examples of labels include, but are not limited to, radioactive label (such as ^{32}P , ^{35}S or ^{125}I), fluorescent label (such as fluorescein isothiocyanate, rhodamine, phycoerythrin, phycocyanin, allophycocyanin, o-phthaldehyde or fluorescamine), and enzymatic label (such as horseradish peroxidase, alkaline phosphatase, or luciferase). The ability of the candidate compound to interact
5 directly or indirectly with perilipin, a fragment thereof, a perilipin fusion protein can be determined by methods known to those of skill in the art. For example, the interaction between a candidate compound and perilipin, a fragment thereof, or a perilipin fusion protein can be determined by flow cytometry, a scintillation assay, immunoprecipitation or western blot analysis.

10 In another embodiment, agents that interact with (*i.e.*, bind to) perilipin (*i.e.*, one or more perilipin isoforms) or a fragment thereof, or a perilipin fusion protein are identified in a cell-free assay system. In accordance with this embodiment, a native or recombinant perilipin (*i.e.*, one or more perilipin isoforms) or a fragment thereof, or a perilipin fusion protein is contacted with a candidate compound or a control compound and the ability of the
15 candidate compound to interact with perilipin, a perilipin fragment, or a perilipin fusion protein is determined. If desired, this assay may be used to screen a plurality (*e.g.* a library) of candidate compounds. It may be desirable to immobilize either perilipin or a candidate/control compound to facilitate separation of complexed from uncomplexed forms of perilipin, as well as to accommodate automation of the assay. Preferably, perilipin or a
20 fragment thereof, or a perilipin fusion protein is first immobilized, by, for example, contacting perilipin or a fragment thereof, or a perilipin fusion protein with an immobilized antibody which specifically recognizes and binds it, or by contacting a purified preparation of perilipin or a fragment thereof, or a perilipin fusion protein with a surface designed to bind proteins. Techniques known to those of skill in the art can be used to immobilize
25 perilipin or a candidate molecule to any suitable vessel (*e.g.*, microtiter plates, test tubes, and microcentrifuge tubes). Perilipin or a fragment thereof, or a perilipin fusion protein may be partially or completely purified (*e.g.*, partially or completely free of other polypeptides) or part of a cell lysate. The ability of the candidate compound to interact with perilipin or a fragment thereof, or a perilipin fusion protein can be determined by methods
30 known to those of skill in the art.

In another embodiment, agents that competitively interact with (*i.e.*, bind to) perilipin (*i.e.*, one or more perilipin isoforms) or a fragment thereof, or a perilipin fusion protein are identified in a competitive binding assay. In accordance with this embodiment, cells expressing perilipin or a fragment thereof, or a perilipin fusion protein are contacted
35 with a candidate compound and a compound known to interact with perilipin (*e.g.*, protein kinase A); the ability of the candidate compound to competitively interact with perilipin or a

fragment thereof, or a perilipin fusion protein is then determined. Alternatively, candidate compounds that competitively interact with (*i.e.*, bind to) perilipin or a fragment thereof, or a perilipin fusion protein are identified in a cell-free assay system by contacting perilipin or a fragment thereof, or a perilipin fusion protein with a candidate compound and a compound known to interact with perilipin. As stated above, the ability of the candidate compound to
5 interact with perilipin or a fragment thereof, or a perilipin fusion protein can be determined by methods known to those of skill in the art. These assays, whether cell-based or cell-free, can be used to screen a plurality (*e.g.*, a library) of candidate compounds.

The assay for compounds that competitively interact perilipin can be conducted in a heterogeneous or homogeneous format. Heterogeneous assays involve anchoring either
10 perilipin or a fragment thereof or a perilipin fusion protein, or a control compound known to interact with perilipin (a "binding partner") onto a solid phase and detecting complexes anchored on the solid phase at the end of the reaction. In homogeneous assays, the entire reaction is carried out in a liquid phase. In either approach, the order of addition of reactants can be varied to obtain different information about the compounds being tested.
15 For example, candidate compounds that interfere with the interaction between perilipin and a binding partner, *e.g.*, by competition, can be identified by conducting the reaction in the presence of the candidate compound (*i.e.*, by adding the candidate compound to the reaction mixture prior to or simultaneously with perilipin and an interactive binding partner). Alternatively, candidate compounds that disrupt preformed complexes, *e.g.* compounds with
20 higher binding constants that displace one of the components from the complex, can be tested by adding the candidate compound to the reaction mixture after complexes have been formed. The various formats are described briefly below.

In a heterogeneous assay system, either perilipin or a fragment thereof, or a perilipin fusion protein, or the binding partner, is anchored onto a solid surface, while the non-
25 anchored species is labeled, either directly or indirectly. In practice, microtiter plates are conveniently utilized. The anchored species can be immobilized by non-covalent or covalent attachments. Non-covalent attachment can be accomplished simply by coating the solid surface with a solution of perilipin or a fragment thereof, or a perilipin fusion protein, or binding partner and drying. Alternatively, an immobilized antibody specific for the
30 species to be anchored can be used to anchor the species to the solid surface. The surfaces can be prepared in advance and stored.

In order to conduct the assay, the partner of the immobilized species is exposed to the coated surface with or without the candidate compound. After the reaction is complete, unreacted components are removed (*e.g.*, by washing) and any complexes formed will
35 remain immobilized on the solid surface. The detection of complexes anchored on the solid surface can be accomplished in a number of ways. Where the non-immobilized species is

pre-labeled, the detection of label immobilized on the surface indicates that complexes were formed. Where the non-immobilized species is not pre-labeled, an indirect label can be used to detect complexes anchored on the surface; *e.g.*, using a labeled antibody specific for the initially non-immobilized species (the antibody, in turn, can be directly labeled or indirectly labeled with a labeled anti-Ig antibody). Depending upon the order of addition of
5 reaction components, candidate compounds which inhibit complex formation or which disrupt preformed complexes can be detected.

Alternatively, the reaction can be conducted in a liquid phase in the presence or absence of the candidate compound, the reaction products separated from unreacted components, and complexes detected; *e.g.*, using an immobilized antibody specific for one
10 of the binding components to anchor any complexes formed in solution, and a labeled antibody specific for the other partner to detect anchored complexes. Again, depending upon the order of addition of reactants to the liquid phase, candidate compounds which inhibit complex or which disrupt preformed complexes can be identified.

In an alternate embodiment of the invention, a homogeneous assay can be used. In
15 this approach, a preformed complex of perilipin, a fragment thereof, or a perilipin fusion protein and the binding partner is prepared in which either the perilipin, a fragment thereof or perilipin fusion protein, or the binding partner is labeled, but the signal generated by the label is quenched due to complex formation (see, *e.g.*, U.S. Patent No. 4,109,496 by Rubenstein which utilizes this approach for immunoassays). The addition of a candidate
20 compound that competes with and displaces one of the species from the preformed complex will result in the generation of a signal above background. In this way, candidate compounds which disrupt perilipin, a fragment thereof or perilipin fusion protein/ binding partner interaction can be identified.

In another embodiment, perilipin (*i.e.*, one or more perilipin isoforms) or a fragment
25 thereof is used as a "bait protein" in a two-hybrid assay or three hybrid assay to identify other proteins that bind to or interact with perilipin (see, *e.g.*, U.S. Patent No. 5,283,317; Zervos et al., 1993, Cell 72:223-232; Madura et al., 1993, J. Biol. Chem. 268:12046-12054; Bartel et al., 1993, Bio/Techniques 14:920-924; Iwabuchi et al., 1993, Oncogene 8:1693-1696; and PCT Publication No. WO 94/10300). As those skilled in the art will appreciate,
30 such binding proteins are also likely to be involved in the propagation of signals by perilipin as, for example, upstream or downstream elements of a signaling pathway involving perilipin.

5.1.2. Assays for Agents that Modulate the Expression or Activity of Perilipin

35 In another embodiment, agents (*i.e.*, candidate compounds) that modulate the expression of perilipin (*i.e.*, one or more perilipin isoforms) are identified by contacting

cells (*e.g.*, cells of prokaryotic origin or eukaryotic origin) expressing perilipin (*i.e.*, one or more perilipin isoforms) with a candidate compound or a control compound (*e.g.*, phosphate buffered saline (PBS)) and determining the expression of perilipin or mRNA encoding perilipin. The level of expression of perilipin or mRNA encoding perilipin in the presence of the candidate compound is compared to the level of expression of perilipin or mRNA
5 encoding perilipin in the absence of the candidate compound (*e.g.*, in the presence of a control compound). The candidate compound can then be identified as a modulator of the expression of perilipin based on this comparison. For example, when expression of perilipin or mRNA encoding perilipin is significantly greater in the presence of the candidate compound than in its absence, the candidate compound is identified as a
10 stimulator of expression of perilipin or mRNA encoding perilipin. Alternatively, when expression of perilipin or mRNA encoding perilipin is significantly less in the presence of the candidate compound than in its absence, the candidate compound is identified as an inhibitor of the expression of perilipin or mRNA encoding perilipin. The level of expression of perilipin or mRNA encoding perilipin can be determined by methods known
15 to those of skill in the art. For example, mRNA expression can be assessed by Northern blot analysis or RT-PCR, and protein levels can be assessed by immunoassays such as western blot analysis and ELISA. In specific embodiments of the invention, the expression of perilipin A, perilipin B, perilipin C, or a combination thereof are measured.

In another embodiment, agents that modulate the activity of perilipin (*i.e.*, one or
20 more perilipin isoforms) are identified by contacting a preparation containing perilipin, or cells (*e.g.*, prokaryotic or eukaryotic cells) expressing perilipin (*i.e.*, one or more perilipin isoforms) with a candidate compound or a control compound and determining the ability of the candidate compound to modulate (*e.g.*, stimulate or inhibit) the activity of perilipin. The activity of perilipin can be assessed by detecting the phosphorylation of perilipin, detecting
25 induction of a cellular signal transduction pathway of perilipin (*e.g.*, intracellular Ca^{2+} , diacylglycerol, IP3, etc.), detecting the activity of an enzyme whose activity is regulated by perilipin (*e.g.*, hormone-sensitive lipase activity), detecting the induction of a reporter gene (*e.g.*, a regulatory element that is responsive to perilipin and is operably linked to a nucleic acid encoding a detectable marker, *e.g.*, luciferase), or detecting a cellular response, for
30 example, lipid hydrolysis (by detecting, *e.g.*, changes in O_2 consumption or glycerol release from fat cells) or distribution of lipid droplets. Techniques known to those of skill in the art and described herein can be used for measuring these activities (see, *e.g.*, Holmes et al., 1997, Methods Enzymol. 286:45-67 and Martinez-Botas et al., 2000, Nature Genetics 26:474-479, each of which is incorporated herein by reference). The candidate compound
35 can then be identified as a modulator of the activity of perilipin by comparing the effects of

the candidate compound to the control compound. Suitable control compounds include phosphate buffered saline (PBS) and normal saline (NS).

In yet another embodiment, agents that modulate the expression, activity or both the expression and activity of perilipin (*i.e.*, one or more perilipin isoforms) are identified in an animal, preferably a mammal. Examples of suitable animals include, but are not limited to, mice, rats, rabbits, monkeys, guinea pigs, dogs and cats. Preferably, the animal used represents a model of obesity such as leptin resistant animals (*e.g.*, *db/db* mice), melanocortin-4 receptor knockout mice (MR-4^{-/-}), leptin-deficient mice (*ob/ob*), tubby mice (tubby protein deficiency), the *fa/fa* (Zucker Diabetic Fatty or ZDF) rat, melanocortin-3 receptor knockout mice, POMC-deficient mice, and *fat/fat* mice (see, *e.g.*, Barsh et al., 2000, *Nature* 404:644-651; Fisher et al., 1999, *Int. J. Obes. Rel. Metab. Disord.* 23 Suppl:54-58; Giridharan, 1998, *Indian J. Med. Res.* 108:225-242; Zhang et al., 1994, *Nature* 372:425-432; Noben-Trauth et al., 1996, *Nature* 380:534-538; Iida et al., 1996, *BBRC* 224:597-604; Phillips et al., 1996, *Nature Genetics* 13:18-19; Chen et al., 2000, *Nature Genetics* 26:97-102; Butler et al., 2000, *Endocrinology* 141:3518-3521; Yawen et al., 1999, *Nature Medicine* 5:1066-1070; and Naggert et al., 1995, *Nature Genetics* 10: 135-142). In accordance with this embodiment, the candidate compound or a control compound is administered (*e.g.*, orally, rectally or parenterally such as subcutaneously, intramuscularly, intraperitoneally or intravenously) to a suitable animal and the effect on the expression, activity or both expression and activity of perilipin is determined. A candidate compound that alters the expression of perilipin can be identified by comparing the level of perilipin or mRNA encoding perilipin in an animal or group of animals treated with a candidate compound with the level of perilipin or mRNA encoding perilipin in an animal or group of animals treated with a control compound. Techniques known to those of skill in the art can be used to determine the mRNA and protein levels, for example, *in situ* hybridization. A candidate compound that alters the activity of perilipin can be identified by assaying the activity of perilipin in animals treated with a control compound and animals treated with the candidate compound. The activity of perilipin can be assessed by detecting the phosphorylation of perilipin, detecting induction of a cellular second messenger of the perilipin (*e.g.*, intracellular Ca²⁺, diacylglycerol, IP₃, etc.), detecting the induction of a reporter gene, or detecting a cellular response (*e.g.*, perilipin distribution on lipid droplets or lipid metabolism such as lipid hydrolysis by detecting changes in O₂ consumption or glycerol release from fat cells). Techniques known to those of skill in the art or described herein can be utilized to detect changes in the activity of perilipin.

5.1.3. Computer Modeling Compounds

Computer modelling and searching technologies permit identification of compounds, or the improvement of already identified compounds, that can modulate perilipin gene expression and/or activity. Having identified such a compound or composition, the active sites or regions are preferably identified. The active site can be
5 identified using methods known in the art including, for example, from the amino acid sequences of peptides, from the nucleotide sequences of nucleic acids, or from study of complexes of the relevant compound or composition with a natural binding partner. For example, chemical or X-ray crystallographic methods can be used to find the active site by finding where on the factor the complexed binding partner is found.

10 The three dimensional geometric structure of the active site is then preferably determined. This can be done by known methods, including X-ray crystallography, which can determine a complete molecular structure. Solid or liquid phase NMR can also be used to determine certain intra-molecular distances within the active site and/or in the binding partner complex. Any other experimental method of structure determination can be used to
15 obtain partial or complete geometric structures. The geometric structures may be measured with a complexed ligand, natural or artificial, which may increase the accuracy of the active site structure determined.

Methods of computer based numerical modelling can be used to complete the structure (*e.g.*, in embodiments wherein an incomplete or insufficiently accurate structure is
20 determined) or to improve its accuracy. Any art recognized modelling method may be used, including, but not limited to, parameterized models specific to particular biopolymers such as proteins or nucleic acids, molecular dynamics models based on computing molecular motions, statistical mechanics models based on thermal ensembles, or combined models. For most types of models, standard molecular force fields, representing the forces between
25 constituent atoms and groups, are necessary, and can be selected from force fields known in physical chemistry. Exemplary forcefields that are known in the art and can be used in such methods include, but are not limited to, the Constant Valence Force Field (CVFF), the AMBER force field and the CHARMM force field. The incomplete or less accurate experimental structures can serve as constraints on the complete and more accurate
30 structures computed by these modeling methods.

Finally, having determined the structure of the active site, either experimentally, by modeling, or by a combination, candidate modulating compounds can be identified by searching databases containing compounds along with information on their molecular structure. Such a search seeks compounds having structures that match the determined
35 active site structure and that interact with the groups defining the active site. Such a search

can be manual, but is preferably computer assisted. These compounds found from this search are potential target or pathway gene product modulating compounds.

Alternatively, these methods can be used to identify improved modulating compounds from an already known modulating compound or binding partner. The composition of the known compound can be modified and the structural effects of modification can be determined using the experimental and computer modelling methods described above applied to the new composition. The altered structure is then compared to the active site structure of the compound to determine if an improved fit or interaction results. In this manner systematic variations in composition, such as by varying side groups, can be quickly evaluated to obtain modified modulating compounds or binding partner of improved specificity or activity.

Further experimental and computer modeling methods useful to identify modulating compounds based upon identification of the active sites of target or pathway gene or gene products and related transduction and transcription factors will be apparent to those of skill in the art.

Examples of molecular modelling systems are the CHARMM and QUANTA programs (Polygen Corporation, Waltham, MA). CHARMM performs the energy minimization and molecular dynamics functions. QUANTA performs the construction, graphic modelling and analysis of molecular structure. QUANTA allows interactive construction, modification, visualization, and analysis of the behavior of molecules with each other.

A number of articles review computer modelling of drugs interactive with specific proteins, such as Rotivinen et al., 1988, *Acta Pharmaceutical Fennica* 97:159-166; Ripka, (June 16, 1988), *New Scientist* 54-57; McKinaly and Rossmann, 1989, *Annu. Rev. Pharmacol. Toxicol.* 29:111-122; Perry and Davies, *OSAR: Quantitative Structure-Activity Relationships in Drug Design* pp. 189-193 (Alan R. Liss, Inc. 1989); Lewis and Dean, 1989 *Proc. R. Soc. Lond.* 236:125-140 and 1-162; and, with respect to a model receptor for nucleic acid components, Askew et al., 1989, *J. Am. Chem. Soc.* 111:1082-1090. Other computer programs that screen and graphically depict chemicals are available from companies such as BioDesign, Inc. (Pasadena, CA.), Allelix, Inc. (Mississauga, Ontario, Canada), and Hypercube, Inc. (Cambridge, Ontario). Although these are primarily designed for application to drugs specific to particular proteins, they can be adapted to design of drugs specific to regions of DNA or RNA, once that region is identified.

Although generally described above with reference to design and generation of compounds which could alter binding, one could also screen libraries of known compounds, including natural products or synthetic chemicals, and biologically active materials, including proteins, for compounds which are inhibitors or activators.

5.2. Perilipin Antagonists

Compounds identified through assays described, above, in Section 5.1, which antagonize perilipin (*i.e.*, one or more perilipin isoforms) by reducing or inhibiting perilipin expression and/or activity levels, can be used in accordance with the invention to prevent, 5 treat or ameliorate one or more signs or symptoms associated with obesity. Further, such compounds can be used to prevent, treat or ameliorate one or more signs or symptoms associated with diabetes. Still further, such compounds can be used to prevent, treat or ameliorate one or more signs or symptoms associated with a lipid metabolic disorder (*e.g.*, a lipodystrophy) or a disorder characterized by lipid accumulation (*e.g.*, atherosclerosis). Still 10 further yet, such compounds can be used to enhance lipid metabolism and increase muscle mass. As discussed in Section 5.1, above, such compounds can include, but are not limited to nucleic acids (*e.g.*, antisense nucleic acids and triple helix molecules), peptides, phosphopeptides, small organic or inorganic molecules, or antibodies (including, for example, polyclonal, monoclonal, human, humanized, anti-idiotypic, chimeric or single 15 chain antibodies, and Fab, F(ab')₂ and Fab expression library fragments, and epitope-binding fragments thereof). In a specific embodiment, an agent that inhibits or reduces perilipin expression and/or activity is a fragment of one or more perilipin isoforms or a derivative thereof, or a fusion protein comprising a fragment of one or more perilipin isoforms or a derivative thereof that prevents endogenously perilipin from functioning normally.

20

5.2.1. Nucleic Acid Antagonists of Perilipin and Pharmaceutical Compositions Based Thereon

The present invention provides perilipin antisense nucleic acids, ribozymes triple helix molecules which target perilipin expression. The present invention further provides pharmaceutical compositions comprising one or more perilipin antisense nucleic acids, 25 triple helix molecules, and/or ribozymes, and pharmaceutically acceptable carriers. Such pharmaceutical compositions can be used for the prevention or treatment of obesity and disorders involving the reduced ability or inability to metabolize lipids. Further, such pharmaceutical compositions can be used for the prevention or treatment of diabetes (*e.g.*, diabetes mellitus). Still further, such pharmaceutical compositions can be used to enhance 30 lipid metabolism and muscle mass in animals, preferably livestock and poultry, and more preferably humans.

5.2.1.1. Perilipin Antisense Nucleic Acids

In a specific embodiment, perilipin expression is inhibited by use of perilipin 35 antisense nucleic acids. The present invention provides the therapeutic or prophylactic use of nucleic acids comprising at least six nucleotides that are antisense to a gene or cDNA

encoding perilipin or a fragment thereof. As used herein, a perilipin "antisense" nucleic acid refers to a nucleic acid capable of hybridizing by virtue of some sequence complementarity to a portion of an RNA (preferably mRNA) encoding perilipin. The antisense nucleic acid may be complementary to a coding and/or noncoding region of an mRNA encoding perilipin. In certain embodiments, a perilipin antisense nucleic acid
 5 inhibits or reduces the expression of one or more perilipin isoforms. In a preferred embodiment, a perilipin antisense nucleic acid inhibits or reduces the expression of perilipin A. Such antisense nucleic acids have utility as compounds that inhibit perilipin expression, and can be used in the prevention or treatment of obesity, lipid metabolic disorders (*e.g.*, lipodystrophies), or disorders characterized by increased lipid accumulation (*e.g.*,
 10 atherosclerosis), and to enhance lipid metabolism and muscle mass.

Representative, non-limiting examples of perilipin antisense molecules include the following:

5' AGGTGAGGCCTTTGTTGACTGCCAT 3' (SEQ ID NO:1)

15 5' CTGCTCAGGGAGGTCTCCATCCAG 3' (SEQ ID NO:2)

Perilipin antisense nucleic acids are oligonucleotides of at least six nucleotides and are preferably oligonucleotides ranging from 6 to about 50 nucleotides. In specific aspects, the perilipin antisense nucleic acids are oligonucleotides of at least 10 nucleotides, at least 15 nucleotides, at 25 nucleotides, at least 50 nucleotides, at least 75 nucleotides, at least 100
 20 nucleotides, at least 150 nucleotides, or at least 200 nucleotides. The perilipin antisense nucleic acids can be DNA or RNA or chimeric mixtures or derivatives or modified versions thereof and can be single-stranded or double-stranded. The perilipin antisense nucleic acids can be modified at the base moiety, sugar moiety, or phosphate backbone. The perilipin antisense nucleic acids may include other appended groups such as peptides; agents that
 25 facilitate transport across the cell membrane (see, *e.g.*, Letsinger et al., 1989, Proc. Natl. Acad. Sci. USA 86:6553-6556; Lemaitre et al., 1987, Proc. Natl. Acad. Sci. 84:648-652; PCT Publication No. WO 88/09810, published December 15, 1988) or blood-brain barrier (see, *e.g.*, PCT Publication No. WO 89/10134, published April 25, 1988); and hybridization-triggered cleavage agents (see, *e.g.*, Krol et al., 1988, BioTechniques 6:958-
 30 976) or intercalating agents (see, *e.g.*, Zon, 1988, Pharm. Res. 5:539-549).

In a preferred aspect of the invention, perilipin antisense nucleic acid is single-stranded DNA. The oligonucleotide may be modified at any position on its structure with substituents generally known in the art.

Perilipin antisense nucleic acids may comprise at least one of the following modified
 35 base moieties: 5-fluorouracil, 5-bromouracil, 5-chlorouracil, 5-iodouracil, hypoxanthine, xantine, 4-acetylcytosine, 5-(carboxyhydroxymethyl) uracil,

5-carboxymethylaminomethyl-2-thiouridine, 5-carboxymethylaminomethyluracil, dihydrouracil, beta-D-galactosylqueosine, inosine, N6-isopentenyladenine, 1-methylguanine, 1-methylinosine, 2,2-dimethylguanine, 2-methyladenine, 2-methylguanine, 3-methylcytosine, 5-methylcytosine, N6-adenine, 7-methylguanine, 5-methylaminomethyluracil, 5-methoxyaminomethyl-2-thiouracil, beta-
 5 D-mannosylqueosine, 5'-methoxycarboxymethyluracil, 5-methoxyuracil, 2-methylthio-N6-isopentenyladenine, uracil-5-oxyacetic acid (v), wybutoxosine, pseudouracil, queosine, 2-thiocytosine, 5-methyl-2-thiouracil, 2-thiouracil, 4-thiouracil, 5-methyluracil, uracil-5-oxyacetic acid methylester, uracil-5-oxyacetic acid (v), 5-methyl-2-thiouracil, 3-(3-amino-3-N-2-carboxypropyl) uracil, (acp3)w, 2,6-diaminopurine, and other base analogs.

10 In another embodiment, perilipin antisense nucleic acids comprises at least one modified sugar moiety, *e.g.*, one of the following sugar moieties: arabinose, 2-fluoroarabinose, xylulose, and hexose.

In another embodiment, perilipin antisense nucleic acids comprises at least one of the following modified phosphate backbones: a phosphorothioate, a phosphorodithioate, a
 15 phosphoramidothioate, a phosphoramidate, a phosphordiamidate, a methylphosphonate, an alkyl phosphotriester, a formacetal, or an analog of formacetal.

In yet another embodiment, perilipin antisense nucleic acids are α -anomeric oligonucleotides. An α -anomeric oligonucleotide forms specific double-stranded hybrids with complementary RNA in which, contrary to the usual β -units, the strands run parallel to
 20 each other (Gautier et al., 1987, Nucl. Acids Res. 15:6625-6641).

Perilipin antisense nucleic acids may be conjugated to another molecule, *e.g.*, a peptide, hybridization triggered cross-linking agent, transport agent, or hybridization-triggered cleavage agent.

Nucleic acids of the invention such as perilipin antisense nucleic acids may be
 25 synthesized by standard methods known in the art, *e.g.*, by use of an automated DNA synthesizer (such as are commercially available from Biosearch, Applied Biosystems, etc.). As examples, phosphorothioate oligonucleotides may be synthesized by the method of Stein et al. (1988, Nucl. Acids Res. 16:3209), and methylphosphonate oligonucleotides can be prepared by use of controlled pore glass polymer supports (Sarin et al., 1988, Proc. Natl.
 30 Acad. Sci. USA 85:7448-7451).

In a specific embodiment, perilipin antisense nucleic acid is administered directly to the cell using techniques known to those of skill in the art such as, for example, microinjection, electroporation, lipofection, and calcium phosphate precipitation. In another specific embodiment, a perilipin antisense nucleic acid of the invention is produced
 35 intracellularly by transcription from an exogenous sequence. For example, a vector can be introduced *in vivo* such that it is taken up by a cell, within which cell the vector or a portion

thereof is transcribed, producing an antisense nucleic acid (RNA) of the invention. Such a vector would contain a sequence encoding the perilipin antisense nucleic acid. Such a vector can remain episomal or become chromosomally integrated (*i.e.*, part of the chromosome), as long as it can be transcribed to produce the desired antisense RNA. Such vectors can be constructed by recombinant DNA technology standard in the art. Vectors
 5 can be plasmid, viral, or others known in the art, used for replication and expression in mammalian cells. Expression of the sequence encoding the perilipin antisense RNA can be by any promoter known in the art to act in an animal cells, preferably mammalian cells, and more preferably human cells. Such promoters can be inducible, constitutive or tissue-specific.

10 Examples of promoters which may be used to regulate perilipin antisense expression include, but are not limited to: the SV40 early promoter region (Bernoist and Chambon, 1981, *Nature* 290:304-310), the promoter contained in the 3' long terminal repeat of Rous sarcoma virus (Yamamoto, et al., 1980, *Cell* 22:787-797), the herpes thymidine kinase promoter (Wagner et al., 1981, *Proc. Natl. Acad. Sci. USA* 78:1441-1445), the regulatory
 15 sequences of the metallothionein gene (Brinster et al., 1982, *Nature* 296:39-42); prokaryotic expression vectors such as the β -lactamase promoter (Villa-Kamaroff et al., 1978, *Proc. Natl. Acad. Sci. USA* 75:3727-3731), or the *tac* promoter (DeBoer et al., 1983, *Proc. Natl. Acad. Sci. USA* 80:21-25); see also "Useful proteins from recombinant bacteria" in *Scientific American*, 1980, 242:74-94; plant expression vectors comprising the nopaline
 20 synthetase promoter region (Herrera-Estrella et al., *Nature* 303:209-213) or the cauliflower mosaic virus 35S RNA promoter (Gardner et al., 1981, *Nucl. Acids Res.* 9:2871), and the promoter of the photosynthetic enzyme ribulose biphosphate carboxylase (Herrera-Estrella et al., 1984, *Nature* 310:115-120); promoter elements from yeast or other fungi such as the Gal 4 promoter, the ADC (alcohol dehydrogenase) promoter, PGK (phosphoglycerol
 25 kinase) promoter, alkaline phosphatase promoter, and the following animal transcriptional control regions, which exhibit tissue specificity and have been utilized in transgenic animals: elastase I gene control region which is active in pancreatic acinar cells (Swift et al., 1984, *Cell* 38:639-646; Ornitz et al., 1986, *Cold Spring Harbor Symp. Quant. Biol.* 50:399-409; MacDonald, 1987, *Hepatology* 7:425-515); insulin gene control region which
 30 is active in pancreatic beta cells (Hanahan, 1985, *Nature* 315:115-122), immunoglobulin gene control region which is active in lymphoid cells (Grosschedl et al., 1984, *Cell* 38:647-658; Adames et al., 1985, *Nature* 318:533-538; Alexander et al., 1987, *Mol. Cell. Biol.* 7:1436-1444), mouse mammary tumor virus control region which is active in testicular, breast, lymphoid and mast cells (Leder et al., 1986, *Cell* 45:485-495), albumin
 35 gene control region which is active in liver (Pinkert et al., 1987, *Genes and Devel.* 1:268-276), alpha-fetoprotein gene control region which is active in liver (Krumlauf et al.,

1985, Mol. Cell. Biol. 5:1639-1648; Hammer et al., 1987, Science 235:53-58; alpha
1-antitrypsin gene control region which is active in the liver (Kelsey et al., 1987, Genes and
Devel. 1:161-171), beta-globin gene control region which is active in myeloid cells
(Mogam et al., 1985, Nature 315:338-340; Kollias et al., 1986, Cell 46:89-94; myelin basic
protein gene control region which is active in oligodendrocyte cells in the brain (Readhead
5 et al., 1987, Cell 48:703-712); myosin light chain-2 gene control region which is active in
skeletal muscle (Sani, 1985, Nature 314:283-286); and gonadotropic releasing hormone
gene control region which is active in the hypothalamus (Mason et al., 1986, Science
234:1372-1378). In a specific embodiment, perilipin antisense expression is regulated by a
regulatory element which is active or preferentially active in steroidogenic cells or
10 adipocytes (e.g., the leptin promoter)

The perilipin antisense nucleic acids of the invention comprise a sequence
complementary to at least a portion of an RNA transcript of a gene encoding perilipin,
preferably a human gene encoding perilipin. However, absolute complementarity, although
preferred, is not required. A sequence "complementary to at least a portion of an RNA," as
15 referred to herein, means a sequence having sufficient complementarity to be able to
hybridize under stringent conditions with the RNA, forming a stable duplex; in the case of
double-stranded perilipin antisense nucleic acids, a single strand of the duplex DNA may
thus be tested, or triplex formation may be assayed. The ability to hybridize will depend on
both the degree of complementarity and the length of the antisense nucleic acid. Generally,
20 the longer the hybridizing nucleic acid, the more base mismatches with an RNA encoding
perilipin it may contain and still form a stable duplex (or triplex, as the case may be). One
skilled in the art can ascertain a tolerable degree of mismatch by use of standard procedures
to determine the melting point of the hybridized complex.

The invention provides pharmaceutical compositions comprising an effective
25 amount of perilipin antisense nucleic acids of the invention and a pharmaceutically
acceptable carrier, as described *infra*. In a specific embodiment, pharmaceutical
compositions comprising one or more perilipin antisense nucleic acids are administered via
liposomes, microparticles, or microcapsules. In various embodiments of the invention, such
compositions may be used to achieve sustained release of perilipin antisense nucleic acids.

30 In another embodiment, the invention provides methods for inhibiting the expression
of a perilipin nucleic acid sequence in a eukaryotic cell (e.g., a steroidogenic cell or
adipocyte) comprising providing the cell with an effective amount of a composition
comprising a perilipin antisense nucleic acid of the invention.

The perilipin antisense nucleic acids can be used to prevent or treat diseases and
35 disorders characterized by aberrant perilipin expression such as lipid metabolic disorders,
weight disorders (e.g., obesity), and diabetes. Further, perilipin antisense nucleic acids can

be used to enhance lipid metabolism and muscle mass. In a preferred embodiment, a single-stranded DNA antisense perilipin oligonucleotide is used.

Pharmaceutical compositions of the invention, comprising an effective amount of one or more perilipin antisense nucleic acids in a pharmaceutically acceptable carrier, can be administered to a subject having or predisposed to lipid metabolic disorders and weight disorders such as obesity. Pharmaceutical compositions of the invention, comprising an effective amount of one or more perilipin antisense nucleic acids in a pharmaceutically acceptable carrier, can be administered to a subject having or predisposed to diabetes (*e.g.*, diabetes mellitus). Pharmaceutical compositions of the invention, comprising an effective amount of one or more perilipin antisense nucleic acids in a pharmaceutically acceptable carrier, can be administered to a subject having or predisposed to disorder characterized by lipid accumulation. The amount of one or more perilipin antisense nucleic acids which will be effective in the prevention or treatment of obesity or diabetes can be determined by standard clinical techniques.

5.2.1.2. Inhibitory Ribozyme and Triple Helix Approaches

In another embodiment, obesity, a lipid metabolic disorder, or a disorder characterized by lipid accumulation, or diabetes may be prevented or treated, and/or lipid metabolism and muscle mass enhanced by decreasing the level of perilipin activity by using gene sequences encoding perilipin in conjunction with well-known gene "knock-out," ribozyme or triple helix methods to decrease gene expression of perilipin. In this approach ribozyme or triple helix molecules are used to modulate the activity, expression or synthesis of the gene encoding perilipin, and thus to prevent or treat obesity a lipid metabolic disorder, or a disorder characterized by lipid accumulation, or diabetes, and/or enhance lipid metabolism and muscle mass. Such molecules may be designed to reduce or inhibit expression of a mutant or non-mutant target gene. Techniques for the production and use of such molecules are well known to those of skill in the art.

Ribozyme molecules designed to catalytically cleave gene mRNA transcripts encoding a perilipin isoform can be used to prevent translation of target gene mRNA and, therefore, expression of the gene product. (See, *e.g.*, PCT International Publication WO90/11364, published October 4, 1990; Sarver et al., 1990, Science 247:1222-1225).

Ribozymes are enzymatic RNA molecules capable of catalyzing the specific cleavage of RNA. (For a review, see Rossi, 1994, Current Biology 4, 469-471). The mechanism of ribozyme action involves sequence specific hybridization of the ribozyme molecule to complementary target RNA, followed by an endonucleolytic cleavage event. The composition of ribozyme molecules must include one or more sequences complementary to the target gene mRNA (*i.e.*, one or more perilipin isoforms), and must

include the well known catalytic sequence responsible for mRNA cleavage. For this sequence, see, *e.g.*, U.S. Patent No. 5,093,246, which is incorporated herein by reference in its entirety.

While ribozymes that cleave mRNA at site specific recognition sequences can be used to destroy mRNAs encoding perilipin, the use of hammerhead ribozymes is preferred. Hammerhead ribozymes cleave mRNAs at locations dictated by flanking regions that form complementary base pairs with the target mRNA. The sole requirement is that the target mRNA have the following sequence of two bases: 5'-UG-3'. The construction and production of hammerhead ribozymes is well known in the art and is described more fully in Myers, 1995, *Molecular Biology and Biotechnology: A Comprehensive Desk Reference*, VCH Publishers, New York, (see especially Figure 4, page 833) and in Haseloff and Gerlach, 1988, *Nature*, 334, 585-591, each of which is incorporated herein by reference in its entirety.

Preferably the ribozyme is engineered so that the cleavage recognition site is located near the 5' end of the mRNA encoding a perilipin isoform, *i.e.*, to increase efficiency and minimize the intracellular accumulation of non-functional mRNA transcripts.

The ribozymes of the present invention also include RNA endoribonucleases (hereinafter "Cech-type ribozymes") such as the one that occurs naturally in *Tetrahymena thermophila* (known as the IVS, or L-19 IVS RNA) and that has been extensively described by Thomas Cech and collaborators (Zaug, et al., 1984, *Science*, 224, 574-578; Zaug and Cech, 1986, *Science*, 231, 470-475; Zaug, et al., 1986, *Nature*, 324, 429-433; published International patent application No. WO 88/04300 by University Patents Inc.; and Been and Cech, 1986, *Cell*, 47, 207-216). The Cech-type ribozymes have an eight base pair active site which hybridizes to a target RNA sequence whereafter cleavage of the target RNA takes place. The invention encompasses those Cech-type ribozymes which target eight base-pair active site sequences that are present in the gene encoding perilipin.

As in the antisense approach, the ribozymes can be composed of modified oligonucleotides (*e.g.*, for improved stability, targeting, etc.) and should be delivered to cells that express perilipin *in vivo*. A preferred method of delivery involves using a DNA construct "encoding" the ribozyme under the control of a strong constitutive pol III or pol II promoter, so that transected cells will produce sufficient quantities of the ribozyme to destroy endogenous mRNA encoding perilipin and inhibit translation. Because ribozymes, unlike antisense molecules, are catalytic, a lower intracellular concentration is required for efficacy.

Endogenous perilipin expression can also be reduced by inactivating or "knocking out" the gene encoding perilipin, or the promoter of such a gene, using targeted homologous recombination (*e.g.*, see Smithies, et al., 1985, *Nature* 317:230-234; Thomas and Capecchi,

1987, Cell 51:503-512; Thompson et al., 1989, Cell 5:313-321; and Zijlstra et al., 1989, Nature 342:435-438, each of which is incorporated by reference herein in its entirety). For example, a mutant gene encoding a non-functional perilipin (or a completely unrelated DNA sequence) flanked by DNA homologous to the endogenous gene (either the coding regions or regulatory regions of the gene encoding the perilipin) can be used, with or
5 without a selectable marker and/or a negative selectable marker, to transfect cells that express the target gene *in vivo*. Insertion of the DNA construct, via targeted homologous recombination, results in inactivation of the target gene. Such approaches are particularly suited in the agricultural field where modifications to ES (embryonic stem) cells can be used to generate animal offspring with an inactive target gene (*e.g.*, see Thomas and
10 Capecchi, 1987 and Thompson, 1989, *supra*). However this approach can be adapted for use in humans provided the recombinant DNA constructs are directly administered or targeted to the required site *in vivo* using appropriate viral vectors.

Alternatively, the endogenous expression of a gene encoding perilipin can be reduced by targeting deoxyribonucleotide sequences complementary to the regulatory
15 region of the gene (*i.e.*, the gene promoter and/or enhancers) to form triple helical structures that prevent transcription of the gene encoding perilipin in target cells in the body. (See generally, Helene, 1991, Anticancer Drug Des., 6(6), 569-584; Helene, et al., 1992, Ann. N.Y. Acad. Sci., 660, 27-36; and Maher, 1992, Bioassays 14(12), 807-815).

Nucleic acid molecules to be used in triplex helix formation for the inhibition of
20 transcription should be single stranded and composed of deoxynucleotides. The base composition of these oligonucleotides must be designed to promote triple helix formation via Hoogsteen base pairing rules, which generally require sizeable stretches of either purines or pyrimidines to be present on one strand of a duplex. Nucleotide sequences may be pyrimidine-based, which will result in TAT and CGC⁺ triplets across the three associated
25 strands of the resulting triple helix. The pyrimidine-rich molecules provide base complementarity to a purine-rich region of a single strand of the duplex in a parallel orientation to that strand. In addition, nucleic acid molecules may be chosen that are purine-rich, for example, contain a stretch of G residues. These molecules will form a triple helix with a DNA duplex that is rich in GC pairs, in which the majority of the purine
30 residues are located on a single strand of the targeted duplex, resulting in GGC triplets across the three strands in the triplex.

Alternatively, the potential sequences that can be targeted for triple helix formation may be increased by creating a so called "switchback" nucleic acid molecule. Switchback molecules are synthesized in an alternating 5'-3', 3'-5' manner, such that they base pair with
35 first one strand of a duplex and then the other, eliminating the necessity for a sizeable stretch of either purines or pyrimidines to be present on one strand of a duplex.

In instances wherein the antisense, ribozyme, or triple helix molecules described herein are utilized to inhibit mutant gene expression, it is possible that the technique may so efficiently reduce or inhibit the transcription (triple helix) or translation (antisense, ribozyme) of mRNA produced by normal gene alleles of perilipin that the situation may arise wherein the concentration of perilipin present may be lower than is necessary for a
5 normal phenotype. In such cases, to ensure that substantially normal levels of activity of a gene encoding perilipin are maintained, gene therapy may be used to introduce into cells nucleic acid molecules that encode and express perilipin that exhibit normal gene activity and that do not contain sequences susceptible to whatever antisense, ribozyme, or triple helix treatments are being utilized. The nucleic acid sequences encoding perilipin can be
10 obtained, *e.g.*, from the GenBank database (*e.g.*, for the nucleic acid sequence encoding human perilipin see GenBank Accession No. AB005293) or a database like it, the literature publications, or by routine cloning and sequencing. Alternatively, in instances whereby the gene encodes an extracellular protein, normal perilipin can be co-administered in order to maintain the requisite level of perilipin activity.

15 Antisense RNA and DNA, ribozyme, and triple helix molecules of the invention may be prepared by any method known in the art for the synthesis of DNA and RNA molecules, as discussed above. These include techniques for chemically synthesizing oligodeoxyribonucleotides and oligoribonucleotides well known in the art such as for example solid phase phosphoramidite chemical synthesis. Alternatively, RNA molecules
20 may be generated by *in vitro* and *in vivo* transcription of DNA sequences encoding the antisense RNA molecule. Such DNA sequences may be incorporated into a wide variety of vectors that incorporate suitable RNA polymerase promoters such as the T7 or SP6 polymerase promoters. Alternatively, antisense cDNA constructs that synthesize antisense RNA constitutively or inducibly, depending on the promoter used, can be introduced stably
25 into cell lines.

5.3. Agonists of Perilipin

Compounds identified through assays described, above, in Section 5.1, which agonize perilipin (*i.e.*, one or more perilipin isoforms) by increasing the expression and/or
30 activity of perilipin can be used in accordance with the invention to prevent, treat or ameliorate one or more signs or symptoms associated with disorders characterized by weight loss (*e.g.*, cachexia and anorexia). Further, agonists of perilipin can be used to increase lipid accumulation in order to increase weight gain. As discussed in Section 5.1, above, such compounds can include, but are not limited to nucleic acids, proteins, peptides,
35 phosphopeptides, small molecules, or antibodies (including, for example, polyclonal,

monoclonal, human, humanized, anti-idiotypic, chimeric or single chain antibodies, and Fab, F(ab')₂ and Fab expression library fragments, and epitope-binding fragments thereof).

In a specific embodiment, one or more perilipin isoforms or a functional fragment thereof are administered to an animal at sufficient dosages such that perilipin activity is increased *in vivo*, *e.g.*, by mimicking the function of perilipin *in vivo*. In another specific
5 embodiment, analogs or derivatives of a perilipin isoform or functional fragment thereof are administered to an animal at sufficient dosages such that perilipin activity is increased *in vivo*, *e.g.*, by mimicking the function of perilipin *in vivo*. In another embodiment, a fusion protein comprising a perilipin isoform, a functional fragment of a perilipin isoform, or a derivative or analog thereof is administered to an animal at sufficient dosages such that
10 perilipin activity is increased *in vivo*, *e.g.*, by mimicking the function of perilipin *in vivo*.

The proteins and peptides which may be used in such methods include synthetic (*e.g.*, recombinant or chemically synthesized) proteins and peptides, as well as naturally occurring proteins and peptides. The proteins and peptides may have both naturally occurring and/or non-naturally occurring amino acid residues (*e.g.*, D-amino acid residues)
15 and/or one or more non-peptide bonds (*e.g.*, imino, ester, hydrazide, semicarbazide, and azo bonds). The proteins or peptides may also contain additional chemical groups (*e.g.*, functional groups) present at the amino and/or carboxy termini, such that, for example, the stability, bioavailability, and/or inhibitory activity of the peptide is enhanced. Exemplary functional groups include hydrophobic groups (*e.g.*, carbobenzoxy, dansyl, and t-
20 butyloxycarbonyl groups) an acetyl group, a 9-fluorenylmethoxy-carbonyl group, and macromolecular carrier groups (*e.g.*, lipid-fatty acid conjugates, polyethylene glycol, or carbohydrates) including peptide groups. In one embodiment, the proteins and peptides used in such methods have one or more amino acid substitutions, additions or deletions that are introduced into the encoded protein or peptide. Mutations can be introduced by standard
25 techniques, such as site-directed mutagenesis and PCR-mediated mutagenesis. Preferably, conservative amino acid substitutions are made at one or more predicted non-essential amino acid residues. A "conservative amino acid substitution" is one in which the amino acid residue is replaced with an amino acid residue having a similar side chain.

Alternatively, mutations can be introduced randomly along all or part of the coding
30 sequence, such as by saturation mutagenesis, and the resultant mutants can be screened for biological activity to identify mutants that retain or antagonize activity. Following mutagenesis, the encoded protein can be expressed recombinantly and the activity of the protein can be determined.

In instances wherein the compound to be administered is a peptide compound, DNA
35 sequences encoding the peptide compound can be directly administered to an animal. Any of the techniques discussed, below, which achieve intracellular administration of

compounds, such as, for example, liposome administration, can be utilized for the administration of such DNA molecules. The DNA molecules can be produced, for example, by well known recombinant techniques.

In instances wherein the disorder involves an aberrant perilipin gene, animals can be treated by gene replacement therapy. One or more copies of a normal perilipin gene or a portion of the gene that directs the production of a normal perilipin with normal perilipin gene function, can be inserted into cells. In a specific embodiment, the nucleic acid sequences are directly administered *in vivo*, where it is expressed to produce the encoded product. This can be accomplished by any of numerous methods known in the art, *e.g.*, by constructing them as part of an appropriate nucleic acid expression vector and administering it so that they become intracellular, *e.g.*, by infection using defective or attenuated retrovirals or other viral vectors (see U.S. Patent No. 4,980,286), or by direct injection of naked DNA, or by use of microparticle bombardment (*e.g.*, a gene gun; Biolistic, Dupont), or coating with lipids or cell-surface receptors or transfecting agents, encapsulation in liposomes, microparticles, or microcapsules, or by administering them in linkage to a peptide which is known to enter the nucleus, by administering it in linkage to a ligand subject to receptor-mediated endocytosis (see, *e.g.*, Wu and Wu, 1987, J. Biol. Chem. 262:4429-4432) (which can be used to target cell types specifically expressing the receptors), etc. In another embodiment, nucleic acid-ligand complexes can be formed in which the ligand comprises a fusogenic viral peptide to disrupt endosomes, allowing the nucleic acid to avoid lysosomal degradation. In yet another embodiment, the nucleic acid can be targeted *in vivo* for cell specific uptake and expression, by targeting a specific receptor (see, *e.g.*, PCT Publications WO 92/06180 dated April 16, 1992 (Wu et al.); WO 92/22635 dated December 23, 1992 (Wilson et al.); WO92/20316 dated November 26, 1992 (Findeis et al.); WO93/14188 dated July 22, 1993 (Clarke et al.), WO 93/20221 dated October 14, 1993 (Young)). Alternatively, the nucleic acid can be introduced intracellularly and incorporated within host cell DNA for expression, by homologous recombination (Koller and Smithies, 1989, Proc. Natl. Acad. Sci. USA 86:8932-8935; and Zijlstra et al., 1989, Nature 342:435-438).

In one embodiment, viral vectors that contain nucleic acids encoding perilipin A, perilipin B, perilipin C, or any combination thereof are used. For example, retroviral vectors can be used. The nucleic acid sequences encoding perilipin to be used in gene therapy are cloned into one or more vectors, which facilitates delivery of the gene into a patient. More detail about retroviral vectors can be found in Boesen et al., 1994, Biotherapy 6:291-302, which describes the use of a retroviral vector to deliver the *mdr1* gene to hematopoietic stem cells in order to make the stem cells more resistant to chemotherapy. Other references illustrating the use of retroviral vectors in gene therapy include: Clowes et

al., 1994, J. Clin. Invest. 93:644-651; Kiem et al., 1994, Blood 83:1467-1473; Salmons and Gunzberg, 1993, Human Gene Therapy 4:129-141; and Grossman and Wilson, 1993, Curr. Opin. in Genetics and Devel. 3:110-114.

Lentiviruses can also be used in gene therapy. Details about the use of lentiviruses in gene therapy can be found, e.g., in Evans et al., 1999, Human Gene Therapy 10:1479-1489, Han et al., 1999, Human Gene Therapy 10:1867-1873; and Zufferey et al., 1997, Nature Biotechnology 15:871-875.

Adenoviruses are other viral vectors that can be used in gene therapy. Adenoviruses are especially attractive vehicles for delivering genes to respiratory epithelia. Adenoviruses naturally infect respiratory epithelia where they cause a mild disease. Other targets for adenovirus-based delivery systems are liver, the central nervous system, endothelial cells, and muscle. Adenoviruses have the advantage of being capable of infecting non-dividing cells. Kozarsky and Wilson, 1993, Current Opinion in Genetics and Development 3:499-503 present a review of adenovirus-based gene therapy. Bout et al., 1994, Human Gene Therapy 5:3-10 demonstrated the use of adenovirus vectors to transfer genes to the respiratory epithelia of rhesus monkeys. Other instances of the use of adenoviruses in gene therapy can be found in Rosenfeld et al., 1991, Science 252:431-434; Rosenfeld et al., 1992, Cell 68:143-155; Mastrangeli et al., 1993, J. Clin. Invest. 91:225-234; PCT Publication WO94/12649; Wang, et al., 1995, Gene Therapy 2:775-783; Chan, 1995, Curr. Opin. Lipidol. 6:335-340; and Oka et al., 2000, Curr. Opin. Lipidol. 11:179-186. In a preferred embodiment, adenovirus vectors are used to express one or more perilipin isoforms or a fragment thereof.

Adeno-associated virus (AAV) has also been proposed for use in gene therapy (Walsh et al., 1993, Proc. Soc. Exp. Biol. Med. 204:289-300; and U.S. Patent No. 5,436,146).

Another approach to gene therapy involves transferring a gene to cells in tissue culture by such methods as electroporation, lipofection, calcium phosphate mediated transfection, or viral infection. Usually, the method of transfer includes the transfer of a selectable marker to the cells. The cells are then placed under selection to isolate those cells that have taken up and are expressing the transferred gene. Those cells are then delivered to a patient.

In this embodiment, the nucleic acid is introduced into a cell prior to administration *in vivo* of the resulting recombinant cell. Such introduction can be carried out by any method known in the art, including but not limited to transfection, electroporation, microinjection, infection with a viral or bacteriophage vector containing the nucleic acid sequences, cell fusion, chromosome-mediated gene transfer, microcell-mediated gene transfer, spheroplast fusion, etc. Numerous techniques are known in the art for the

introduction of foreign genes into cells (see, *e.g.*, Loeffler and Behr, 1993, Meth. Enzymol. 217:599-618; Cohen et al., 1993, Meth. Enzymol. 217:618-644; and Cline, 1985, Pharmac. Ther. 29:69-92) and may be used in accordance with the present invention, provided that the necessary developmental and physiological functions of the recipient cells are not disrupted. The technique should provide for the stable transfer of the nucleic acid to the cell, so that
5 the nucleic acid is expressible by the cell and preferably heritable and expressible by its cell progeny.

The resulting recombinant cells can be delivered to a patient by various methods known in the art. Recombinant blood cells (*e.g.*, hematopoietic stem or progenitor cells) are preferably administered intravenously. The amount of cells envisioned for use depends on
10 the desired effect, patient state, etc., and can be determined by one skilled in the art.

Cells into which a nucleic acid can be introduced for purposes of gene therapy encompass any desired, available cell type, and include but are not limited to steroidogenic cells, adipocytes, epithelial cells, endothelial cells, keratinocytes, fibroblasts, muscle cells, hepatocytes; blood cells such as T lymphocytes, B lymphocytes, monocytes, macrophages,
15 neutrophils, eosinophils, megakaryocytes, and granulocytes; and various stem or progenitor cells, in particular hematopoietic stem or progenitor cells, *e.g.*, as obtained from bone marrow, umbilical cord blood, peripheral blood, fetal liver, etc. In a preferred embodiment, the cells used for gene therapy are adipocytes or steroidogenic cells. In another preferred embodiment, the cell used for gene therapy is autologous to the patient.

20 In one embodiment in which recombinant cells are used in gene therapy, nucleic acid sequences encoding perilipin are introduced into the cells such that they are expressible by the cells or their progeny, and the recombinant cells are then administered *in vivo* for therapeutic effect. In a specific embodiment, stem or progenitor cells are used. Any stem and/or progenitor cells which can be isolated and maintained *in vitro* can potentially be used
25 in accordance with this embodiment of the present invention (see *e.g.* PCT Publication WO 94/08598, dated April 28, 1994; Stemple and Anderson, 1992, Cell 71:973-985; Rheinwald, 1980, Meth. Cell Bio. 21A:229; and Pittelkow and Scott, 1986, Mayo Clinic Proc. 61:771).

In one embodiment, the nucleic acid to be introduced for purposes of gene therapy comprises a constitutive promoter operably linked to the coding region of perilipin, such
30 that the expression of the nucleic acid is constitutive. In another embodiment, the nucleic acid to be introduced for purposes of gene therapy comprises an inducible promoter operably linked to the coding region of perilipin, such that expression of the nucleic acid is controllable by controlling the presence or absence of the appropriate inducer of transcription. Examples of constitutive or inducible promoters described herein or known to
35 those of ordinary skill in the art can be used to regulate expression of nucleic acid molecules

encoding perilipin or a fragment thereof, or a perilipin fusion protein for purposes of gene therapy.

5.4. Antagonists or Agonists of Perilipin

Described hereinbelow are compounds which, depending on the specific application
5 for which they are utilized, can either function as antagonists or agonists of one or more perilipin isoforms.

In certain embodiments, compounds which function as antagonists or agonists of
perilipin specifically bind to (*i.e.*, bind with little or no cross-reactivity to related antigens as
determined by immunoassays well-known to those skilled in the art) and/or recognize
10 perilipin A, perilipin B, perilipin C, or any combination thereof. In other embodiments,
compounds which function as antagonists or agonists of perilipin specifically modulate the
expression and/or activity of perilipin A, perilipin B, perilipin C, or any combination thereof.

5.4.1. Antibodies

15 Antibodies functioning as antagonists or agonists can be utilized to prevent, treat or
ameliorate one or more signs or symptoms associated with weight associated disorders (*e.g.*,
obesity), lipid metabolic disorders (*e.g.*, lipodystrophies), disorders characterized by lipid
accumulation (*e.g.*, atherosclerosis) or diabetes. Depending on the specific antibody, the
antibody can function as an antagonist or agonist.

20 An antibody that functions as an antagonist of one or more perilipin isoforms is an
antibody which specifically binds to and interferes with the action of one or more perilipin
isoforms. For example, such an antibody could specifically bind a perilipin isoform in a
manner which does not activate the perilipin isoform but which disrupts the ability of the
perilipin isoform to bind to a natural ligand. Such antibodies include, but are not limited to,
25 polyclonal, monoclonal, humanized, human, Fab fragments, single chain antibodies,
chimeric antibodies, and the like.

An antibody that functions as an agonist of one or more perilipin isoforms is an
antibody which specifically binds to a perilipin and, by binding, serves to, either directly or
indirectly, activate a function of one or more perilipin isoforms. For example, an antibody
30 can bind to a perilipin isoform in a manner which causes the perilipin isoform to function
as though an endogenous ligand was binding, thus activating, for example, a signal
transduction pathway. Such antibodies, include but are not limited to polyclonal,
monoclonal, human, humanized, FAb fragments, single chain antibodies, chimeric
antibodies, and the like.

35 In one embodiment, the antibodies used as antagonists or agonists of perilipin
specifically recognize and/or bind to perilipin A, perilipin B, perilipin C, or any combination

thereof. Where fragments of the antibody are used, the smallest inhibitory fragment which binds to perilipin is preferred. For example, peptides having an amino acid sequence corresponding to the domain of the variable region of the antibody that binds to perilipin can be used. Such peptides can be synthesized chemically or produced via recombinant DNA technology using methods well known in the art (*e.g.*, see Creighton, 1983, *supra*; and
 5 Sambrook et al., 1989, *supra*). Alternatively, single chain antibodies, such as neutralizing antibodies, which bind to intracellular epitopes can also be administered. Such single chain antibodies can be administered, for example, by expressing nucleotide sequences encoding single-chain antibodies within the target cell population by utilizing, for example, techniques such as those described in Marasco et al. (Marasco, W. et al., 1993, Proc. Natl.
 10 Acad. Sci. USA 90:7889-7893).

In certain embodiments, any commercially available antibody that specifically binds to one or more perilipin isoforms or a fragment thereof can be used in accordance with the invention. An example of an antibody that specifically binds to perilipin is the polyclonal guinea pig anti-perilipin antibody available commercially by Research Diagnostics, Inc.
 15 (Flanders, NJ). In certain other embodiments, commercially available antibodies that specifically bind to one or more perilipin isoforms or a fragment thereof are not used in accordance with the invention.

5.4.1.1. Production of Antibodies

20 One or more perilipin isoforms or fragments thereof may be used as an immunogen to generate antibodies which immunospecifically bind to one or more perilipin isoforms. Such immunogens can be isolated by any convenient means known to those of skill in the art. Antibodies of the invention include, but are not limited to polyclonal, monoclonal, bispecific, human, humanized or chimeric antibodies, single chain antibodies, Fab
 25 fragments and F(ab') fragments, fragments produced by a Fab expression library, anti-idiotypic (anti-Id) antibodies, and epitope-binding fragments of any of the above. The term "antibody" as used herein refers to immunoglobulin molecules and immunologically active portions of immunoglobulin molecules, *i.e.*, molecules that contain an antigen binding site that specifically binds an antigen. The immunoglobulin molecules can be of
 30 any class (*e.g.*, IgG, IgE, IgM, IgD and IgA) or subclass of immunoglobulin molecule.

The anti-perilipin antibodies include analogs and derivatives that are either modified, *i.e.*, by the covalent attachment of any type of molecule as long as such covalent attachment that does not impair immunospecific binding. For example, but not by way of limitation, the derivatives and analogs of the antibodies include those that have been further
 35 modified, *e.g.*, by glycosylation, acetylation, pegylation, phosphorylation, amidation, derivatization by known protecting/blocking groups, proteolytic cleavage, linkage to a

cellular ligand or other protein, etc. Any of numerous chemical modifications may be carried out by known techniques, including, but not limited to specific chemical cleavage, acetylation, formylation, etc. Additionally, the analog or derivative may contain one or more non-classical amino acids.

In the production of antibodies, screening for the desired antibody can be accomplished by techniques known in the art, *e.g.* ELISA (enzyme-linked immunosorbent assay). For example, to select antibodies which recognize a specific region of perilipin, one may assay generated hybridomas for a product which binds to a perilipin fragment containing such domain. For selection of an antibody that specifically binds, *e.g.*, to perilipin A but which does not specifically bind to (or binds less avidly to) perilipin B or C, one can select on the basis of positive binding to perilipin A and a lack of binding to (or reduced binding to) perilipin B or C. Thus, the present invention provides an antibody (preferably a monoclonal antibody) that binds with greater affinity (preferably at least 2-fold, more preferably at least 5-fold still more preferably at least 10-fold greater affinity) to a specific perilipin isoform than to a different isoform or isoforms of perilipin.

Polyclonal antibodies which may be used in the methods of the invention are heterogeneous populations of antibody molecules derived from the sera of immunized animals. Unfractionated immune serum can also be used. Various procedures known in the art may be used for the production of polyclonal antibodies to a perilipin isoform or a fragment thereof. In a particular embodiment, rabbit polyclonal antibodies to an epitope of a perilipin isoform can be obtained. For example, for the production of polyclonal or monoclonal antibodies, various host animals can be immunized by injection with the native or a synthetic (*e.g.*, recombinant) version of a perilipin isoform or fragment thereof, including but not limited to rabbits, mice, rats, etc. Various adjuvants may be used to enhance the immunological response, depending on the host species, including, but not limited to, complete or incomplete Freund's adjuvant, a mineral gel such as aluminum hydroxide, surface active substance such as lysolecithin, pluronic polyol, a polyanion, a peptide, an oil emulsion, keyhole limpet hemocyanin, dinitrophenol, and an adjuvant such as BCG (bacille Calmette-Guerin) or corynebacterium parvum. Additional adjuvants are also well known in the art.

For preparation of monoclonal antibodies (mAbs) directed toward a perilipin isoform or fragment thereof, any technique which provides for the production of antibody molecules by continuous cell lines in culture may be used. For example, the hybridoma technique originally developed by Kohler and Milstein (1975, Nature 256:495-497), as well as the trioma technique, the human B-cell hybridoma technique (Kozbor et al., 1983, Immunology Today 4:72), and the EBV-hybridoma technique to produce human monoclonal antibodies (Cole et al., 1985, in Monoclonal Antibodies and Cancer Therapy,

Alan R. Liss, Inc., pp. 77-96). Such antibodies may be of any immunoglobulin class including IgG, IgM, IgE, IgA, IgD and any subclass thereof. The hybridoma producing the mAbs of the invention may be cultivated *in vitro* or *in vivo*. In an additional embodiment of the invention, monoclonal antibodies can be produced in germ-free animals utilizing known technology (PCT/US90/02545, incorporated herein by reference).

5 The monoclonal antibodies include but are not limited to human monoclonal antibodies and chimeric monoclonal antibodies (*e.g.*, human-mouse chimeras). A chimeric antibody is a molecule in which different portions are derived from different animal species, such as those having a human immunoglobulin constant region and a variable region derived from a murine mAb. (See, *e.g.*, Cabilly et al., U.S. Patent No. 4,816,567; and Boss
10 et al., U.S. Patent No. 4,816,397, which are incorporated herein by reference in their entirety.) Humanized antibodies are antibody molecules from non-human species having one or more complementarily determining regions (CDRs) from the non-human species and a framework region from a human immunoglobulin molecule. (See, *e.g.*, Queen, U.S. Patent No. 5,585,089, which is incorporated herein by reference in its entirety.)

15 Chimeric and humanized monoclonal antibodies can be produced by recombinant DNA techniques known in the art, for example using methods described in PCT Publication No. WO 87/02671; European Patent Application 184,187; European Patent Application 171,496; European Patent Application 173,494; PCT Publication No. WO 86/01533; U.S. Patent No. 4,816,567; European Patent Application 125,023; Better et al., 1988, Science
20 240:1041-1043; Liu et al., 1987, Proc. Natl. Acad. Sci. USA 84:3439-3443; Liu et al., 1987, J. Immunol. 139:3521-3526; Sun et al., 1987, Proc. Natl. Acad. Sci. USA 84:214-218; Nishimura et al., 1987, Canc. Res. 47:999-1005; Wood et al., 1985, Nature 314:446-449; and Shaw et al., 1988, J. Natl. Cancer Inst. 80:1553-1559; Morrison, 1985, Science 229:1202-1207; Oi et al., 1986, Bio/Techniques 4:214; U.S. Patent 5,225,539;
25 Jones et al., 1986, Nature 321:552-525; Verhoeyan et al. (1988) Science 239:1534; and Beidler et al., 1988, J. Immunol. 141:4053-4060.

Completely human antibodies are particularly desirable for therapeutic treatment of human subjects. Such antibodies can be produced using transgenic mice which are incapable of expressing endogenous immunoglobulin heavy and light chains genes, but
30 which can express human heavy and light chain genes. The transgenic mice are immunized in the normal fashion with a selected antigen, *e.g.*, all or a portion of a perilipin isoform. Monoclonal antibodies directed against the antigen can be obtained using conventional hybridoma technology. The human immunoglobulin transgenes harbored by the transgenic mice rearrange during B cell differentiation, and subsequently undergo class switching and
35 somatic mutation. Thus, using such a technique, it is possible to produce therapeutically useful IgG, IgA, IgM and IgE antibodies. For an overview of this technology for producing

human antibodies, see Lonberg and Huszar (1995, Int. Rev. Immunol. 13:65-93). For a detailed discussion of this technology for producing human antibodies and human monoclonal antibodies and protocols for producing such antibodies, *see, e.g.*, U.S. Patent 5,625,126; U.S. Patent 5,633,425; U.S. Patent 5,569,825; U.S. Patent 5,661,016; and U.S. Patent 5,545,806. In addition, companies such as Abgenix, Inc. (Freemont, CA) and
5 Genpharm (San Jose, CA) can be engaged to provide human antibodies directed against a selected antigen using technology similar to that described above.

Completely human antibodies which recognize a selected epitope can be generated using a technique referred to as "guided selection." In this approach a selected non-human monoclonal antibody, *e.g.*, a mouse antibody, is used to guide the selection of a completely
10 human antibody recognizing the same epitope. (Jespers et al. (1994) Bio/technology 12:899-903).

The anti-perilipin antibodies can also be generated using various phage display methods known in the art. In phage display methods, functional antibody domains are displayed on the surface of phage particles which carry the polynucleotide sequences
15 encoding them. In a particular, such phage can be utilized to display antigen binding domains expressed from a repertoire or combinatorial antibody library (*e.g.*, human or murine). Phage expressing an antigen binding domain that binds the antigen of interest can be selected or identified with antigen, *e.g.*, using labeled antigen or antigen bound or captured to a solid surface or bead. Phage used in these methods are typically filamentous
20 phage including fd and M13 binding domains expressed from phage with Fab, Fv or disulfide stabilized Fv antibody domains recombinantly fused to either the phage gene III or gene VIII protein. Phage display methods that can be used to make the antibodies of the present invention include those disclosed in Brinkman et al., J. Immunol. Methods 182:41-50 (1995); Ames et al., J. Immunol. Methods 184:177-186 (1995); Kettleborough et al., Eur.
25 J. Immunol. 24:952-958 (1994); Persic et al., Gene 187 9-18 (1997); Burton et al., Advances in Immunology 57:191-280 (1994); PCT Application No. PCT/GB91/01134; PCT Publications WO 90/02809; WO 91/10737; WO 92/01047; WO 92/18619; WO 93/11236; WO 95/15982; WO 95/20401; and U.S. Patent Nos. 5,698,426; 5,223,409; 5,403,484; 5,580,717; 5,427,908; 5,750,753; 5,821,047; 5,571,698; 5,427,908; 5,516,637;
30 5,780,225; 5,658,727; 5,733,743 and 5,969,108; each of which is incorporated herein by reference in its entirety.

As described in the above references, after phage selection, the antibody coding regions from the phage can be isolated and used to generate whole antibodies, including human antibodies, or any other desired antigen binding fragment, and expressed in any
35 desired host, including mammalian cells, insect cells, plant cells, yeast, and bacteria, *e.g.*, as described in detail below. For example, techniques to recombinantly produce Fab, Fab' and

F(ab')₂ fragments can also be employed using methods known in the art such as those disclosed in PCT publication WO 92/22324; Mullinax et al., *BioTechniques* 12(6):864-869 (1992); and Sawai et al., *AJRI* 34:26-34 (1995); and Better et al., *Science* 240:1041-1043 (1988) (said references incorporated by reference in their entireties).

Examples of techniques which can be used to produce single-chain Fvs and
5 antibodies include those described in U.S. Patents 4,946,778 and 5,258,498; Huston et al., *Methods in Enzymology* 203:46-88 (1991); Shu et al., *PNAS* 90:7995-7999 (1993); and Skerra et al., *Science* 240:1038-1040 (1988).

The invention further provides for the use of bispecific antibodies, which can be made by methods known in the art. Traditional production of full length bispecific
10 antibodies is based on the coexpression of two immunoglobulin heavy chain-light chain pairs, where the two chains have different specificities (Milstein et al., 1983, *Nature* 305:537-539). Because of the random assortment of immunoglobulin heavy and light chains, these hybridomas (quadromas) produce a potential mixture of 10 different antibody molecules, of which only one has the correct bispecific structure. Purification of the correct
15 molecule, which is usually done by affinity chromatography steps, is rather cumbersome, and the product yields are low. Similar procedures are disclosed in WO 93/08829, published May 13, 1993, and in Traunecker et al., 1991, *EMBO J.* 10:3655-3659.

According to a different and more preferred approach, antibody variable domains with the desired binding specificities (antibody-antigen combining sites) are fused to
20 immunoglobulin constant domain sequences. The fusion preferably is with an immunoglobulin heavy chain constant domain, comprising at least part of the hinge, CH₂, and CH₃ regions. It is preferred to have the first heavy-chain constant region (CH₁) containing the site necessary for light chain binding, present in at least one of the fusions. DNAs encoding the immunoglobulin heavy chain fusions and, if desired, the
25 immunoglobulin light chain, are inserted into separate expression vectors, and are co-transfected into a suitable host organism. This provides for great flexibility in adjusting the mutual proportions of the three polypeptide fragments in embodiments when unequal ratios of the three polypeptide chains used in the construction provide the optimum yields. It is, however, possible to insert the coding sequences for two or all three polypeptide chains
30 in one expression vector when the expression of at least two polypeptide chains in equal ratios results in high yields or when the ratios are of no particular significance.

In a preferred embodiment of this approach, the bispecific antibodies are composed of a hybrid immunoglobulin heavy chain with a first binding specificity in one arm, and a hybrid immunoglobulin heavy chain-light chain pair (providing a second binding
35 specificity) in the other arm. It was found that this asymmetric structure facilitates the separation of the desired bispecific compound from unwanted immunoglobulin chain

combinations, as the presence of an immunoglobulin light chain in only one half of the bispecific molecule provides for a facile way of separation. This approach is disclosed in WO 94/04690 published March 3, 1994. For further details for generating bispecific antibodies see, for example, Suresh et al., *Methods in Enzymology*, 1986, 121:210.

The invention provides functionally active fragments, derivatives or analogs of the anti-perilipin immunoglobulin molecules. Functionally active means that the fragment, derivative or analog is able to elicit anti-anti-idiotypic antibodies (*i.e.*, tertiary antibodies) that recognize the same antigen that is recognized by the antibody from which the fragment, derivative or analog is derived. Specifically, in a preferred embodiment the antigenicity of the idiotype of the immunoglobulin molecule may be enhanced by deletion of framework and CDR sequences that are C-terminal to the CDR sequence that specifically recognizes the antigen. To determine which CDR sequences bind the antigen, synthetic peptides containing the CDR sequences can be used in binding assays with the antigen by any binding assay method known in the art.

The present invention provides antibody fragments such as F(ab')₂ fragments and Fab fragments. Antibody fragments which recognize specific epitopes may be generated by known techniques. F(ab')₂ fragments consist of the variable region, the light chain constant region and the CH1 domain of the heavy chain and are generated by pepsin digestion of the antibody molecule. Fab fragments are generated by reducing the disulfide bridges of the F(ab')₂ fragments. The invention also provides heavy chain and light chain dimers of the antibodies of the invention, or any minimal fragment thereof such as Fvs or single chain antibodies (SCAs) (*e.g.*, as described in U.S. Patent 4,946,778; Bird, 1988, *Science* 242:423-42; Huston et al., 1988, *Proc. Natl. Acad. Sci. USA* 85:5879-5883; and Ward et al., 1989, *Nature* 334:544-54), or any other molecule with the same specificity as the antibody of the invention. Single chain antibodies are formed by linking the heavy and light chain fragments of the Fv region via an amino acid bridge, resulting in a single chain polypeptide. Techniques for the assembly of functional Fv fragments in *E. coli* may be used (Skerra et al., 1988, *Science* 242:1038-1041).

In other embodiments, the invention provides fusion proteins of the anti-perilipin antibodies (or functionally active fragments thereof), for example in which the antibody is fused via a covalent bond (*e.g.*, a peptide bond), at either the N-terminus or the C-terminus to an amino acid sequence of another protein (or portion thereof, preferably at least 10, 20 or 50 amino acid portion of the protein) that is not the antibody. Preferably, the immunoglobulin or fragment thereof is covalently linked to the other protein at the N-terminus of the constant domain. Such fusion proteins may facilitate purification or increase half-life *in vivo*.

5.4.1.2. Expression of Antibodies

The anti-perilipin antibodies can be produced by any method known in the art for the synthesis of antibodies, in particular, by chemical synthesis or by recombinant expression, and are preferably produced by recombinant expression technique.

Recombinant expression of antibodies, or fragments, derivatives or analogs thereof, requires construction of a nucleic acid that encodes the antibody. If the nucleotide sequence of the antibody is known, a nucleic acid encoding the antibody may be assembled from chemically synthesized oligonucleotides (*e.g.*, as described in Kutmeier et al., 1994, BioTechniques 17:242), which, briefly, involves the synthesis of overlapping oligonucleotides containing portions of the sequence encoding antibody, annealing and ligation of those oligonucleotides, and then amplification of the ligated oligonucleotides by PCR.

Alternatively, the nucleic acid encoding the antibody may be obtained by cloning the antibody. If a clone containing the nucleic acid encoding the particular antibody is not available, but the sequence of the antibody molecule is known, a nucleic acid encoding the antibody may be obtained from a suitable source (*e.g.*, an antibody cDNA library, or cDNA library generated from any tissue or cells expressing the antibody) by PCR amplification using synthetic primers hybridizable to the 3' and 5' ends of the sequence or by cloning using an oligonucleotide probe specific for the particular gene sequence.

If an antibody molecule that specifically recognizes a particular perilipin isoform is not available (or a source for a cDNA library for cloning a nucleic acid encoding such an antibody), antibodies specific for a particular perilipin isoform may be generated by any method known in the art, for example, by immunizing an animal, such as a rabbit, to generate polyclonal antibodies or, more preferably, by generating monoclonal antibodies. Alternatively, a clone encoding at least the Fab portion of the antibody may be obtained by screening Fab expression libraries (*e.g.*, as described in Huse et al., 1989, Science 246:1275-1281) for clones of Fab fragments that bind the specific antigen or by screening antibody libraries (See, *e.g.*, Clackson et al., 1991, Nature 352:624; and Hane et al., 1997 Proc. Natl. Acad. Sci. USA 94:4937).

Once a nucleic acid encoding at least the variable domain of the antibody molecule is obtained, it may be introduced into a vector containing the nucleotide sequence encoding the constant region of the antibody molecule (see, *e.g.*, PCT Publication WO 86/05807; PCT Publication WO 89/01036; and U.S. Patent No. 5,122,464). Vectors containing the complete light or heavy chain for co-expression with the nucleic acid to allow the expression of a complete antibody molecule are also available. Then, the nucleic acid encoding the antibody can be used to introduce the nucleotide substitution(s) or deletion(s) necessary to substitute (or delete) the one or more variable region cysteine residues

participating in an intrachain disulfide bond with an amino acid residue that does not contain a sulfhydryl group. Such modifications can be carried out by any method known in the art for the introduction of specific mutations or deletions in a nucleotide sequence, for example, but not limited to, chemical mutagenesis, *in vitro* site directed mutagenesis (Hutchinson et al., 1978, J. Biol. Chem. 253:6551), PCT based methods, etc.

5 In addition, techniques developed for the production of "chimeric antibodies" (Morrison et al., 1984, Proc. Natl. Acad. Sci. 81:851-855; Neuberger et al., 1984, Nature 312:604-608; Takeda et al., 1985, Nature 314:452-454) by splicing genes from a mouse antibody molecule of appropriate antigen specificity together with genes from a human antibody molecule of appropriate biological activity can be used. As described *supra*, a
10 chimeric antibody is a molecule in which different portions are derived from different animal species, such as those having a variable region derived from a murine mAb and a human antibody constant region, *e.g.*, humanized antibodies.

Once a nucleic acid encoding an anti-perilipin antibody molecule has been obtained, the vector for the production of the antibody molecule may be produced by recombinant
15 DNA technology using techniques well known in the art. Thus, methods for preparing the protein of the invention by expressing nucleic acid containing the antibody molecule sequences are described herein. Methods which are well known to those skilled in the art can be used to construct expression vectors containing an antibody molecule coding sequences and appropriate transcriptional and translational control signals. These methods
20 include, for example, *in vitro* recombinant DNA techniques, synthetic techniques, and *in vivo* genetic recombination. See, for example, the techniques described in Sambrook et al. (1990, Molecular Cloning, A Laboratory Manual, 2d Ed., Cold Spring Harbor Laboratory, Cold Spring Harbor, NY) and Ausubel et al. (eds., 1998, Current Protocols in Molecular Biology, John Wiley & Sons, NY).

25 The expression vector is transferred to a host cell by conventional techniques and the transected cells are then cultured by conventional techniques to produce an antibody of the invention.

The host cells used to express a recombinant antibody of the invention may be either bacterial cells such as *Escherichia coli*, or, preferably, eukaryotic cells, especially for the
30 expression of whole recombinant antibody molecule. In particular, mammalian cells such as Chinese hamster ovary cells (CHO), in conjunction with a vector such as the major intermediate early gene promoter element from human cytomegalovirus is an effective expression system for antibodies (Foecking et al., 198, Gene 45:101; Cockett et al., 1990, Bio/Technology 8:2).

35 A variety of host-expression vector systems may be utilized to express an anti-perilipin antibody molecule. Such host-expression systems represent vehicles by which the

coding sequences of interest may be produced and subsequently purified, but also represent cells which may, when transformed or transected with the appropriate nucleotide coding sequences, express the antibody molecule of the invention *in situ*. These include, but are not limited to, microorganisms such as bacteria (*e.g.*, *E. coli*, *B. subtilis*) transformed with recombinant bacteriophage DNA, plasmid DNA or cosmid DNA expression vectors
 5 containing antibody coding sequences; yeast (*e.g.*, *Saccharomyces*, *Pichia*) transformed with recombinant yeast expression vectors containing antibody coding sequences; insect cell systems infected with recombinant virus expression vectors (*e.g.*, baculovirus) containing the antibody coding sequences; plant cell systems infected with recombinant virus expression vectors (*e.g.*, cauliflower mosaic virus, CaMV; tobacco mosaic virus,
 10 TMV) or transformed with recombinant plasmid expression vectors (*e.g.*, Ti plasmid) containing antibody coding sequences; or mammalian cell systems (*e.g.*, COS, CHO, BHK, 293, 3T3 cells) harboring recombinant expression constructs containing promoters derived from the genome of mammalian cells (*e.g.*, metallothionein promoter) or from mammalian viruses (*e.g.*, the adenovirus late promoter; the vaccinia virus 7.5K promoter).

15 In bacterial systems, a number of expression vectors may be advantageously selected depending upon the use intended for the antibody molecule being expressed. For example, when a large quantity of such a protein is to be produced, for the generation of pharmaceutical compositions comprising an antibody molecule, vectors which direct the expression of high levels of fusion protein products that are readily purified may be
 20 desirable. Such vectors include, but are not limited, to the *E. coli* expression vector pUR278 (Ruther et al., 1983, EMBO J. 2:1791), in which the antibody coding sequence may be ligated individually into the vector in frame with the *lac Z* coding region so that a fusion protein is produced; pIN vectors (Inouye & Inouye, 1985, Nucleic Acids Res. 13:3101-3109; and Van Heeke & Schuster, 1989, J. Biol. Chem. 24:5503-5509); and the
 25 like. pGEX vectors may also be used to express foreign polypeptides as fusion proteins with glutathione S-transferase (GST). In general, such fusion proteins are soluble and can easily be purified from lysed cells by adsorption and binding to a matrix glutathione-agarose beads followed by elution in the presence of free glutathione. The pGEX vectors are designed to include thrombin or factor Xa protease cleavage sites so that the cloned target
 30 gene product can be released from the GST moiety.

In an insect system, *Autographa californica* nuclear polyhedrosis virus (AcNPV) is used as a vector to express foreign genes. The virus grows in *Spodoptera frugiperda* cells. The antibody coding sequence may be cloned individually into non-essential regions (for example the polyhedrin gene) of the virus and placed under control of an AcNPV promoter
 35 (for example the polyhedrin promoter). In mammalian host cells, a number of viral-based expression systems (*e.g.*, an adenovirus expression system) may be utilized.

As discussed above, a host cell strain may be chosen which modulates the expression of the inserted sequences, or modifies and processes the gene product in the specific fashion desired. Such modifications (*e.g.*, glycosylation) and processing (*e.g.*, cleavage) of protein products may be important for the function of the protein.

For long-term, high-yield production of recombinant antibodies, stable expression is preferred. For example, cells lines that stably express an antibody of interest can be produced by transfecting the cells with an expression vector comprising the nucleotide sequence of the antibody and the nucleotide sequence of a selectable (*e.g.*, neomycin or hygromycin), and selecting for expression of the selectable marker. Such engineered cell lines may be particularly useful in screening and evaluation of compounds that interact directly or indirectly with the antibody molecule.

The expression levels of the antibody molecule can be increased by vector amplification (for a review, see Bebbington and Hentschel, The use of vectors based on gene amplification for the expression of cloned genes in mammalian cells in DNA cloning, Vol.3. (Academic Press, New York, 1987)). When a marker in the vector system expressing antibody is amplifiable, increase in the level of inhibitor present in culture of host cell will increase the number of copies of the marker gene. Since the amplified region is associated with the antibody gene, production of the antibody will also increase (Crouse et al., 1983, Mol. Cell. Biol. 3:257).

The host cell may be co-transfected with two expression vectors of the invention, the first vector encoding a heavy chain derived polypeptide and the second vector encoding a light chain derived polypeptide. The two vectors may contain identical selectable markers which enable equal expression of heavy and light chain polypeptides. Alternatively, a single vector may be used which encodes both heavy and light chain polypeptides. In such situations, the light chain should be placed before the heavy chain to avoid an excess of toxic free heavy chain (Proudfoot, 1986, Nature 322:52; and Kohler, 1980, Proc. Natl. Acad. Sci. USA 77:2197). The coding sequences for the heavy and light chains may comprise cDNA or genomic DNA.

Once the antibody molecule of the invention has been recombinantly expressed, it may be purified by any method known in the art for purification of an antibody molecule, for example, by chromatography (*e.g.*, ion exchange chromatography, affinity chromatography such as with protein A or specific antigen, and sizing column chromatography), centrifugation, differential solubility, or by any other standard technique for the purification of proteins.

Alternatively, any fusion protein may be readily purified by utilizing an antibody specific for the fusion protein being expressed. For example, a system described by Janknecht et al. allows for the ready purification of non-denatured fusion proteins expressed

in human cell lines (Janknecht et al., 1991, Proc. Natl. Acad. Sci. USA 88:8972-897). In this system, the gene of interest is subcloned into a vaccinia recombination plasmid such that the open reading frame of the gene is translationally fused to an amino-terminal tag consisting of six histidine residues. The tag serves as a matrix binding domain for the fusion protein. Extracts from cells infected with recombinant vaccinia virus are loaded onto
 5 Ni^{2+} nitriloacetic acid-agarose columns and histidine-tagged proteins are selectively eluted with imidazole-containing buffers.

5.4.1.3. Conjugated Antibodies

In a preferred embodiment, anti-perilipin antibodies or fragments thereof are
 10 conjugated to a diagnostic or therapeutic moiety. The antibodies can be used for diagnosis or to determine the efficacy of a given treatment regimen. Detection can be facilitated by coupling the antibody to a detectable substance. Examples of detectable substances include various enzymes, prosthetic groups, fluorescent materials, luminescent materials, bioluminescent materials, radioactive nuclides, positron emitting metals (for use in positron
 15 emission tomography), and nonradioactive paramagnetic metal ions. See generally U.S. Patent No. 4,741,900 for metal ions which can be conjugated to antibodies for use as diagnostics according to the present invention. Suitable enzymes include horseradish peroxidase, alkaline phosphatase, beta-galactosidase, or acetylcholinesterase; suitable prosthetic groups include streptavidin, avidin and biotin; suitable fluorescent materials
 20 include umbelliferone, fluorescein, fluorescein isothiocyanate, rhodamine, dichlorotriazinylamine fluorescein, dansyl chloride and phycoerythrin; suitable luminescent materials include luminol; suitable bioluminescent materials include luciferase, luciferin, and aequorin; and suitable radioactive nuclides include ^{125}I , ^{131}I , ^{111}In , and ^{99}Tc .

Anti-perilipin antibodies or fragments thereof can be conjugated to a therapeutic
 25 agent or drug moiety to modify a given biological response. The therapeutic agent or drug moiety is not to be construed as limited to classical chemical therapeutic agents. For example, the drug moiety may be a protein or polypeptide possessing a desired biological activity. Such proteins may include, for example, a toxin such as abrin, ricin A, pseudomonas exotoxin, or diphtheria toxin; a protein such as tumor necrosis factor,
 30 interferon- α , interferon- β , nerve growth factor, platelet derived growth factor, tissue plasminogen activator, a thrombotic agent or an anti-angiogenic agent, *e.g.*, angiostatin or endostatin; or, a biological response modifier such as a lymphokine, interleukin-1 (IL-1), interleukin-2 (IL-2), interleukin-6 (IL-6), granulocyte macrophage colony stimulating factor (GM-CSF), granulocyte colony stimulating factor (G-CSF), nerve growth factor (NGF) or
 35 other growth factor.

Techniques for conjugating such therapeutic moiety to antibodies are well known, see, *e.g.*, Arnon et al., "Monoclonal Antibodies For Immunotargeting Of Drugs In Cancer Therapy", in *Monoclonal Antibodies And Cancer Therapy*, Reisfeld et al. (eds.), pp. 243-56 (Alan R. Liss, Inc. 1985); Hellstrom et al., "Antibodies For Drug Delivery", in *Controlled Drug Delivery* (2nd Ed.), Robinson et al. (eds.), pp. 623-53 (Marcel Dekker, Inc. 1987);
 5 Thorpe, "Antibody Carriers Of Cytotoxic Agents In Cancer Therapy: A Review", in *Monoclonal Antibodies '84: Biological And Clinical Applications*, Pinchera et al. (eds.), pp. 475-506 (1985); "Analysis, Results, And Future Prospective Of The Therapeutic Use Of Radiolabeled Antibody In Cancer Therapy", in *Monoclonal Antibodies For Cancer Detection And Therapy*, Baldwin et al. (eds.), pp. 303-16 (Academic Press 1985), and
 10 Thorpe et al., "The Preparation And Cytotoxic Properties Of Antibody-Toxin Conjugates", *Immunol. Rev.*, 62:119-58 (1982).

Alternatively, an antibody can be conjugated to a second antibody to form an antibody heteroconjugate as described by Segal in U.S. Patent No. 4,676,980.

An antibody with or without a therapeutic moiety conjugated to it can be used as a
 15 therapeutic that is administered alone or in combination with cytotoxic factor(s) and/or cytokine(s).

5.5. Methods of Treating Disorders Related to Body Weight and/or Inappropriate Regulation of Lipid Metabolism

The invention provides for the prevention, treatment or amelioration of one or more
 20 signs or symptoms associated with weight disorders such as obesity, disorders characterized by inappropriate lipid metabolism (*e.g.*, lipodystrophies), disorders characterized by lipid accumulation (*e.g.*, atherosclerosis), and diabetes by the administration of one or more pharmaceutical compositions comprising one or more compounds that modulate the expression and/or activity of one or more perilipin isoforms. In a preferred embodiment,
 25 pharmaceutical compositions are administered to a subject (*i.e.*, an animal) to prevent, treat or ameliorate one or more signs or symptoms associated with obesity. In another preferred embodiment, pharmaceutical compositions are administered to a subject (*i.e.*, an animal) to prevent, treat, or ameliorate one or more signs or symptoms associated with diabetes (*e.g.*, type 1 or 2 diabetes mellitus). In accordance with these embodiments, the pharmaceutical
 30 composition comprises one or more compounds which antagonize the activity and/or expression of one or more perilipin isoforms, and a pharmaceutical acceptable carrier.

In another preferred embodiment, pharmaceutical compositions are administered to a subject (*i.e.*, an animal, preferably a mammal, and mor preferably a human) to prevent, treat
 35 or ameliorate one or more signs or symptoms associated with disorders characterized by weight loss such as cachexia, bulimia, and anorexia. In accordance with this embodiment, the pharmaceutical composition comprises one or more compounds which agonize the

activity and/or expression of one or more perilipin isoforms, and a pharmaceutically acceptable carrier.

In specific embodiments, a subject is administered a composition of the present invention in an amount effective for enhancing lipid metabolism, or an amount effective for increasing muscle mass, or an amount to increase body weight, or an amount to reduce body fat, or an amount effective to increase insulin secretion, or an amount effective to reduce insulin resistance, or an amount effective to reduce glucose intolerance, or an effective amount for the prevention, treatment or amelioration of one or more signs or symptoms associated with obesity, disorders characterized by inappropriate lipid metabolism, disorders characterized by lipid accumulation, or diabetes. In other specific embodiments, a subject is administered a composition of the present invention in an amount effective for the treatment, prevention or amelioration of one or more signs or symptoms associated with disorders characterized by weight loss such as cachexia and anorexia, or an amount effective to increase body fat, or an amount effective to increase body weight, or an amount effective for enhancing lipid accumulation.

The present invention provides methods for treating, preventing or ameliorating one or more signs or symptoms associated with weight disorders and/or inappropriate lipid metabolism that involve localized effects in fats. Thus, the methods of the invention are less likely to have adverse side effects that are observed with other targets for the treatment of weight disorders such as obesity.

In a specific embodiment, a compound identified in accordance with the methods of the invention for use in the prevention, treatment or amelioration of one or more signs or symptoms associated with a weight disorder (*e.g.*, obesity), a disorder characterized by inappropriate lipid metabolism, a disorder characterized by lipid accumulation, and diabetes is a compound which was not known or previously used in the prevention, treatment or amelioration of such disorders. In another embodiment, a compound identified in accordance with the methods of the invention for use in the prevention, treatment or amelioration of one or more signs or symptoms associated with a weight disorder (*e.g.*, obesity), a disorder characterized by inappropriate lipid metabolism, a disorder characterized by lipid accumulation, and diabetes is a compound which preferentially affects the expression or activity of one perilipin isoform and not the other two isoforms. In another embodiment, a compound identified in accordance with the methods of the invention for use in the prevention, treatment or amelioration of one or more signs or symptoms associated with a weight disorder (*e.g.*, obesity), a disorder characterized by inappropriate lipid metabolism, a disorder characterized by lipid accumulation, and diabetes is a compound which preferentially affects perilipin A expression or activity.

5.6. Methods of Enhancing Livestock and Poultry

The present invention provides methods and compositions for the enhancement of body weight and/or performance of an animal, preferably to companion animals (*e.g.*, dogs, cats and horses), livestock (*e.g.*, cows, horses, and pigs) and poultry (*e.g.*, chickens and turkeys). The present invention also provides methods and compositions for the prevention
5 or treatment of a weight disorder such as obesity in an animal, preferably to companion animals (*e.g.*, dogs, cats and horses), livestock (*e.g.*, cows, horses, and pigs) and poultry (*e.g.*, chickens and turkeys). The present invention also provides methods and compositions for the prevention, treatment or amelioration of one or more signs or symptoms associated with a disorder characterized by inappropriate lipid metabolism, a disorder characterized by
10 lipid accumulation, or diabetes in an animal, preferably to companion animals (*e.g.*, dogs, cats and horses), livestock (*e.g.*, cows, horses, and pigs) and poultry (*e.g.*, chickens and turkeys). The present invention further relates to methods and compositions for the improvement of the health of an animal, preferably to companion animals (*e.g.*, dogs, cats and horses), livestock (*e.g.*, cows, horses, and pigs) and poultry (*e.g.*, chickens and turkeys).

15 In one embodiment, pharmaceutical compositions comprising one or more antagonists of one or more perilipin isoforms are administered to livestock or poultry to enhance their body weight and/or performance. In another embodiment, pharmaceutical compositions comprising one or more antagonists of one or more perilipin isoforms are administered to livestock or poultry to enhance lipid metabolism and/or increase muscle
20 mass. In another embodiment, pharmaceutical compositions comprising one or more antagonists of one or more perilipin isoforms are administered to livestock or poultry to treat, prevent or ameliorate one or more signs or symptoms associated with obesity, a lipid metabolic disorder, a disorder characterized by lipid accumulation, or diabetes. In yet another embodiment, pharmaceutical compositions comprising one or more agonists of one
25 or more perilipin isoforms are administered to livestock or poultry to treat, prevent or ameliorate one or more signs or symptoms associated with a weight disorder characterized by weight loss such as cachexia or anorexia.

5.7. Pharmaceutical Formulations and Routes of Administration

30 The invention provides methods of treatment (and prophylaxis) comprising administering to a subject an effective amount of a compound of the invention. In a preferred aspect, the compound is substantially purified (*e.g.*, substantially free from substances that limit its effect or produce undesired side-effects). The subject is preferably an animal, including but not limited to animals such as cows, pigs, horses, chickens, cats,
35 dogs, etc., and is preferably a mammal, and most preferably a human. In a specific

embodiment, poultry or a non-human mammal is the subject or animal. In another specific embodiment, the subject or animal is a human.

Formulations and methods of administration that can be employed when the compound comprises a nucleic acid are described above; additional appropriate formulations and routes of administration are described below.

5 Various delivery systems are known and can be used to administer a compound of the invention, *e.g.*, encapsulation in liposomes, microparticles, microcapsules, recombinant cells capable of expressing the compound, receptor-mediated endocytosis (see, *e.g.*, Wu and Wu, 1987, J. Biol. Chem. 262:4429-4432), construction of a nucleic acid as part of a retroviral or other vector, etc. Methods of introduction can be enteral or parenteral and
10 include, but are not limited to, intradermal, intramuscular, intraperitoneal, intravenous, subcutaneous, intranasal, epidural, and oral routes. The compounds may be administered by any convenient route, for example by infusion or bolus injection, by absorption through epithelial or mucocutaneous linings (*e.g.*, oral mucosa, rectal and intestinal mucosa, etc.) and may be administered together with other biologically active agents. Administration can
15 be systemic or local.

In a specific embodiment, it may be desirable to administer the pharmaceutical compositions of the invention locally to the area in need of treatment; this may be achieved, for example, and not by way of limitation, by local infusion during surgery, topical application, *e.g.*, by injection, by means of a catheter, or by means of an implant, said
20 implant being of a porous, non-porous, or gelatinous material, including membranes, such as sialastic membranes, or fibers.

In another embodiment, the compound can be delivered in a vesicle, in particular a liposome (see Langer, 1990, Science 249:1527-1533; Treat et al., in Liposomes in the Therapy of Infectious Disease and Cancer, Lopez-Berestein and Fidler (eds.), Liss, New
25 York, pp. 353-365 (1989); Lopez-Berestein, *ibid.*, pp. 317-327; see generally *ibid.*)

In yet another embodiment, the compound can be delivered in a sustained or controlled release system. In one embodiment, a pump may be used (see Langer, *supra*; Sefton, 1987, CRC Crit. Ref. Biomed. Eng. 14:201; Buchwald et al., 1980, Surgery 88:507; Saudek et al., 1989, N. Engl. J. Med. 321:574). In another embodiment, polymeric
30 materials can be used (see Medical Applications of Controlled Release, Langer and Wise (eds.), CRC Pres., Boca Raton, Florida (1974); Controlled Drug Bioavailability, Drug Product Design and Performance, Smolen and Ball (eds.), Wiley, New York (1984); Ranger and Peppas, J., 1983, Macromol. Sci. Rev. Macromol. Chem. 23:61; Levy et al., 1985, Science 228:190; During et al., 1989, Ann. Neurol. 25:351; and Howard et al., 1989,
35 J. Neurosurg. 71:105). In yet another embodiment, a sustained or controlled release system can be placed in proximity of the therapeutic target, *i.e.*, adipose tissue, thus requiring only

a fraction of the systemic dose (see, *e.g.*, Goodson, in Medical Applications of Controlled Release, *supra*, vol. 2, pp. 115-138 (1984)).

Other controlled release systems are discussed in the review by Langer (1990, Science 249:1527-1533).

In a specific embodiment where the compound of the invention is a nucleic acid
5 encoding a protein, the nucleic acid can be administered *in vivo* to promote expression of its encoded protein, by constructing it as part of an appropriate nucleic acid expression vector and administering it so that it becomes intracellular, *e.g.*, by use of a retroviral vector (see U.S. Patent No. 4,980,286), or by direct injection, or by use of microparticle bombardment (*e.g.*, a gene gun; Biolistic, Dupont), or coating with lipids or cell-surface receptors or
10 transfecting agents, or by administering it in linkage to a homeobox-like peptide which is known to enter the nucleus (see *e.g.*, Joliot et al., 1991, Proc. Natl. Acad. Sci. USA 88:1864-1868), etc. Alternatively, a nucleic acid can be introduced intracellularly and incorporated within host cell DNA for expression, by homologous recombination.

The present invention also provides pharmaceutical compositions. Such
15 compositions comprise a prophylactically or therapeutically effective amount of a compound, and a pharmaceutically acceptable carrier. In a specific embodiment, the term "pharmaceutically acceptable" means approved by a regulatory agency of the Federal or a state government or listed in the U.S. Pharmacopeia or other generally recognized pharmacopeia for use in animals, and more particularly in humans. The term "carrier"
20 refers to a diluent, adjuvant, excipient, or vehicle with which the therapeutic is administered. Such pharmaceutical carriers can be sterile liquids, such as water and oils, including those of petroleum, animal, vegetable or synthetic origin, such as peanut oil, soybean oil, mineral oil, sesame oil and the like. Water is a preferred carrier when the pharmaceutical composition is administered intravenously. Saline solutions and aqueous
25 dextrose and glycerol solutions can also be employed as liquid carriers, particularly for injectable solutions. Suitable pharmaceutical excipients include starch, glucose, lactose, sucrose, gelatin, malt, rice, flour, chalk, silica gel, sodium stearate, glycerol monostearate, talc, sodium chloride, dried skim milk, glycerol, propylene, glycol, water, ethanol and the like. The composition, if desired, can also contain minor amounts of wetting or emulsifying
30 agents, or pH buffering agents. These compositions can take the form of solutions, suspensions, emulsion, tablets, pills, capsules, powders, sustained-release formulations and the like. The composition can be formulated as a suppository, with traditional binders and carriers such as triglycerides. Oral formulation can include standard carriers such as pharmaceutical grades of mannitol, lactose, starch, magnesium stearate, sodium saccharine,
35 cellulose, magnesium carbonate, etc. Examples of suitable pharmaceutical carriers are described in "Remington's Pharmaceutical Sciences" by E.W. Martin. Such compositions

will contain a therapeutically effective amount of the compound, preferably in purified form, together with a suitable amount of carrier so as to provide the form for proper administration to the subject. The formulation should suit the mode of administration. In a preferred embodiment, the pharmaceutical compositions of the invention are sterile.

In a preferred embodiment, the composition is formulated in accordance with
5 routine procedures as a pharmaceutical composition adapted for intravenous administration to human beings. Typically, compositions for intravenous administration are solutions in sterile isotonic aqueous buffer. Where necessary, the composition may also include a solubilizing agent and a local anesthetic such as lidocaine to ease pain at the site of the injection. Generally, the ingredients are supplied either separately or mixed together in unit
10 dosage form, for example, as a dry lyophilized powder or water free concentrate in a hermetically sealed container such as an ampoule or sachette indicating the quantity of active agent. Where the composition is to be administered by infusion, it can be dispensed with an infusion bottle containing sterile pharmaceutical grade water or saline. Where the composition is administered by injection, an ampoule of sterile water for injection or saline
15 can be provided so that the ingredients may be mixed prior to administration.

The compounds of the invention can be formulated as neutral or salt forms. Pharmaceutically acceptable salts include those formed with free amino groups such as those derived from hydrochloric, phosphoric, acetic, oxalic, tartaric acids, etc., and those formed with free carboxyl groups such as those derived from sodium, potassium,
20 ammonium, calcium, ferric hydroxides, isopropylamine, triethylamine, 2-ethylamino ethanol, histidine, procaine, etc.

Toxicity and therapeutic efficacy of compounds can be determined by standard pharmaceutical procedures in cell cultures or experimental animals, *e.g.*, for determining the LD_{50} (the dose lethal to 50% of the population) and the ED_{50} (the dose therapeutically
25 effective in 50% of the population). The dose ratio between toxic and therapeutic effects is the therapeutic index and it can be expressed as the ratio LD_{50}/ED_{50} . Compounds which exhibit large therapeutic indices are preferred. While compounds that exhibit toxic side effects can be used, care should be taken to design a delivery system that targets such compounds to the site of affected tissue in order to minimize potential damage to uninfected
30 cells and, thereby, reduce side effects.

The data obtained from the cell culture assays and animal studies can be used in formulating a range of dosage for use in humans and the frequency of administration of a dosage. The dosage of such compounds lies preferably within a range of circulating concentrations that include the ED_{50} with little or no toxicity. The dosage can vary within
35 this range depending upon the dosage form employed and the route of administration utilized. For any compound used in a method of the invention, the therapeutically effective

dose can be estimated initially from cell culture assays. A dose can be formulated in animal models to achieve a circulating plasma concentration range that includes the IC_{50} (*i.e.*, the concentration of the candidate compound which achieves a half-maximal inhibition of symptoms) as determined in cell culture. Such information can be used to more accurately determine useful doses in humans. Levels in plasma can be measured, for example, by high performance liquid chromatography.

A suitable dosage ranges for intravenous administration are generally about 20-500 micrograms of active compound per kilogram body weight. Suppositories generally contain active ingredient in the range of 0.5% to 10% by weight; oral formulations preferably contain 10% to 95% active ingredient.

10 For antibodies, the preferred dosage is 0.1 mg/kg to 125 mg/kg (more preferably, 0.1 mg/kg to 75 mg/kg, 0.1 mg/kg to 50 mg/kg, 0.1 mg/kg to 25 mg/kg, 0.1 mg/kg to 20 mg/kg, 0.1 mg/kg to 15 mg/kg, 0.1 mg/kg to 10 mg/kg, 0.1 mg/kg to 5 mg/kg, 0.1 mg/kg to 2.5 mg/kg, or 0.1 to 1 mg/kg). Generally, partially human antibodies and fully human antibodies have a longer half-life within the human body than other antibodies.

15 Accordingly, lower dosages and less frequent administration is often possible. Modifications such as lipidation can be used to stabilize antibodies and to enhance uptake and tissue penetration. A method for lipidation of antibodies is described by Cruikshank et al., 1997, J. Acquired Immune Deficiency Syndromes and Human Retrovirology 14;193.

A therapeutically effective amount of a protein or polypeptide (*i.e.*, an effective dose or effective dosage) ranges from about 0.001 to 30 mg/kg of body weight, preferably about 20 0.01 to 25 mg/kg body weight, more preferably about 0.1 to 20 mg/kg body weight, and even more preferably about 1 to 10 mg/kg, 2 to 9 mg/kg, 3 to 8 mg/kg, 4 to 7 mg/kg, or 5 to 6 mg/kg body weight.

The skilled artisan will appreciate that certain factors may influence the dosage required to effectively treat a subject, including but not limited to the severity of the disease 25 or disorder, previous treatments, the general health and/or age of the subject, and other diseases present. Moreover, treatment of a subject with a therapeutically effective amount of a protein, polypeptide or antibody can include a single treatment or, preferably, can include a series of treatments. In a preferred example, a subject is treated with antibody, 30 protein, or polypeptide in the range of between about 0.1 to 20 mg/kg body weight one time per week for between about 1 to 10 weeks, preferably between 2 to 8 weeks, more preferably between about 3 to 7 weeks, and even more preferably for about 4, 5, or 6 weeks. It will also be appreciated that the effective dosage of antibody, protein or polypeptide used for treatment may increase or decrease over the course of a particular treatment. Changes in 35 dosage may also be apparent to one skilled in the art from the results of diagnostic assays as described herein.

It is understood that appropriate doses of small molecule agents depends upon a number of factors within the ken of the ordinary skilled physician, veterinarian, or researcher. The dose(s) of the small molecule will vary, for example, depending upon the identity, size, and condition of the subject or sample being treated, further depending upon the route by which the composition is to be administered, if applicable, and the effect which the practitioner desires the small molecule to have upon the nucleic acid or polypeptide of the invention. Exemplary doses include milligram or microgram amounts of the small molecule per kilogram of the subject or sample weight (*e.g.*, about 1 microgram per kilogram to about 500 milligrams per kilogram, about 1 microgram per kilogram to about 250 milligrams per kilogram, about 1 microgram per kilogram to about 100 milligrams per kilogram, about 1 microgram per kilogram to about 50 milligrams per kilogram, about 1 microgram per kilogram to about 25 milligrams per kilogram, about 1 microgram per kilogram to about 10 milligrams per kilogram, about 1 microgram per kilogram to about 5 milligrams per kilogram, about 1 microgram per kilogram to about 1 microgram per kilogram, about 1 microgram per kilogram to about 500 micograms per kilogram, about 1 microgram per kilogram to about 250 micograms per kilogram, about 1 microgram per kilogram to about 100 micograms per kilogram, about 1 microgram per kilogram to about 50 micograms per kilogram, about 1 microgram per kilogram to about 25 micograms per kilogram, or about 1 microgram per kilogram to about 10 micograms per kilogram). It will be understood that appropriate doses of a small molecule depend upon the potency of the small molecule with respect to the expression or activity to be modulated. Such appropriate doses may be determined using the assays known in the art or described herein. When one or more small molecules is to be administered to an animal (*e.g.*, a human) in order to modulate the expression or activity of one or more perilipin isoforms, a physician, veterinarian, or researcher may, for example, prescribe relatively low dose at first, subsequently increasing the dose until an appropriate response is obtained. Further, it is understood that the specific dose level for any particular animal subject will depend upon a variety of factors including the activity of the specific compound employed, the age, body weight, general health, gender, and diet of the subject, the time of administration, the route of administration, the rate of excretion, any drug combination, and the degree of expression or activity of one or more perilipin isoforms to be modulated.

The invention also provides a pharmaceutical pack or kit comprising one or more containers filled with one or more of the ingredients of the pharmaceutical compositions of the invention. Optionally associated with such container(s) can be a notice in the form prescribed by a governmental agency regulating the manufacture, use or sale of pharmaceuticals or biological products, which notice reflects (a) approval by the agency of manufacture, use or sale for human administration, (b) directions for use, or both.

5.8. Methods of Assessing Therapeutic Utility

The present invention also provides assays for use in drug discovery in order to identify or verify the efficacy of compounds for treatment or prevention of weight disorders (*e.g.*, obesity, cachexia, and anorexia), lipid metabolic disorders, disorders characterized by lipid accumulation, and diabetes. Candidate compounds can be assayed for their ability to modulate perilipin expression levels in a subject having a weight disorder towards levels found in subjects free from such disorders. Compounds able to restore the expression levels of one or more perilipin isoforms in a subject having a weight disorder characterized by weight loss towards levels found in subjects free from the weight disorder can be used as lead compounds for further drug discovery, or used therapeutically. Further, compounds able to reduce the expression levels of one or more perilipin isoforms in a subject having a weight disorder characterized by weight gain (*e.g.*, obesity) towards levels found in subjects free the weight disorder can be used as lead compounds for further drug discovery, or used therapeutically. Perilipin isoform expression can be assayed by immunoassays, gel electrophoresis followed by visualization, detection of perilipin phosphorylation, detection of perilipin activity, or any other method taught herein or known to those skilled in the art. Such assays can be used to screen candidate drugs, in clinical monitoring or in drug development, where abundance of a perilipin isoform can serve as a surrogate marker for clinical disease.

In various specific embodiments, *in vitro* assays can be carried out with cells representative of cell types involved in a disorder, to determine if a compound has a desired effect upon such cell types. For example, steroidogenic cells and adipocytes from an animal having a weight disorder such as obesity can be used to determine if a compound has a desired effect upon such cells.

Compounds for use in therapy can be tested in suitable animal model systems prior to testing in humans, including but not limited to rats, mice, chicken, cows, monkeys, rabbits, etc. For *in vivo* testing, prior to administration to humans, any animal model system known in the art may be used. Examples of animal models of weight disorders include, but are not limited to, model of obesity such as leptin resistant animals (*e.g.*, db/db mice), melanocortin-4 receptor knockout mice (MR-4^{-/-}), leptin-deficient mice (ob/ob), tubby mice (tubby protein deficiency), the fa/fa (Zucker Diabetic Fatty or ZDF) rat, melanocortin-3 receptor knockout mice, POMC-deficient mice, and fat/fat mice (see, *e.g.*, Barsh et al., 2000, Nature 404:644-651; Fisher et al., 1999, Int. J. Obes. Rel. Metab. Disord. 23 Suppl:54-58; Giridharan, 1998, Indian J. Med. Res. 108:225-242; Zhang et al., 1994, Nature 372:425-432; Noben-Trauth et al., 1996, Nature 380:534-538; Iida et al., 1996, BBRC 224:597-604; Phillips et al., 1996, Nature Genetics 13:18-19; Chen et al., 2000, Nature Genetics 26:97-102; Butler et al., 2000, Endocrinology 141:3518-3521; Yawen et al., 1999,

Nature Medicine 5:1066-1070; and Naggert et al., 1995, Nature Genetics 10: 135-142). It is also apparent to the skilled artisan that, based upon the present disclosure, transgenic animals can be produced with "knock-out" mutations of the gene or genes encoding perilipin. A "knock-out" mutation of a gene is a mutation that causes the mutated gene to not be expressed, or expressed in an aberrant form or at a low level, such that the activity
5 associated with the gene product is nearly or entirely absent. Preferably, the transgenic animal is a mammal, more preferably, the transgenic animal is a mouse.

In one embodiment, candidate compounds that modulate the level or expression of perilipin isoforms are identified or verified in human subjects having a weight disorder such as obesity, cachexia or anorexia, a lipid metabolic disorder, a disorder characterized by lipid
10 accumulation, or diabetes. In accordance with this embodiment, a candidate compound or a control compound is administered to the human subject, and the effect of a test compound on perilipin expression is determined by analyzing the expression of perilipin or the mRNA encoding the same in a biological sample (*e.g.*, serum or plasma). A candidate compound that alters the expression of one or more perilipin isoforms can be identified by comparing
15 the level of one or more perilipin isoforms or mRNA encoding the same in a subject or group of subjects treated with a control compound to that in a subject or group of subjects treated with a candidate compound. Alternatively, alterations in the expression of one or more perilipin isoforms can be identified by comparing the level of one or more perilipin isoforms or mRNA encoding the same in a subject or group of subjects before and after the
20 administration of a candidate compound. Techniques known to those of skill in the art can be used to obtain the biological sample and analyze the mRNA or protein expression.

In another embodiment, candidate compounds that modulate the activity of perilipin are identified or verified in human subjects having a weight disorder, a lipid metabolic disorder, a disorder characterized by lipid accumulation, or diabetes. In accordance with
25 this embodiment, a candidate compound or a control compound is administered to the human subject, and the effect of a candidate compound on the activity of perilipin is determined. A candidate compound that alters the activity of one or more perilipin isoforms can be identified by comparing biological samples from subjects treated with a control compound to samples from subjects treated with the candidate compound. Alternatively,
30 alterations in the activity of one or more perilipin isoforms can be identified by comparing the activity of one or more perilipin isoforms in a subject or group of subjects before and after the administration of a candidate compound. The activity of perilipin can be assessed by detecting the phosphorylation of perilipin, detecting in a biological sample (*e.g.*, serum or plasma) induction of a cellular signal transduction pathway of perilipin (*e.g.*, intracellular
35 Ca^{2+} , diacylglycerol, IP3, etc.), detecting the activity of an enzyme whose activity is regulated by perilipin (*e.g.*, hormone-sensitive lipase activity), detecting the induction of a

reporter gene, or a cellular response, for example, lipid metabolism (*e.g.*, by detecting changes in levels triacylglycerol, nonesterified fatty acids, or β -hydroxybutyrate).

Techniques known to those of skill in the art can be used to detect changes in the phosphorylation of perilipin, changes in the induction of a second messenger of perilipin or changes in a cellular response. For example, RT-PCR can be used to detect changes in the
5 induction of a cellular second messenger and immunoprecipitation followed by western blot analysis can be used to detect changes in the phosphorylation of perilipin.

In a preferred embodiment, a candidate compound that changes the level or expression of one or more perilipin isoforms towards levels detected in control subjects (*e.g.*, humans free from a weight disorder) is selected for further testing or therapeutic use.

10 In another preferred embodiment, a candidate compound that changes the activity of one or more perilipin isoforms towards the activity found in control subjects (*e.g.*, humans free from a weight disorder) is selected for further testing or therapeutic use.

In another embodiment, candidate compounds that reduce the severity of one or more signs or symptoms associated with a weight disorder are identified in human subjects
15 having a weight disorder. In accordance with this embodiment, a candidate compound or a control compound is administered to a human subject having a weight disorder, and the effect of a candidate compound on one or more signs or symptoms of the weight disorder is determined. A candidate compound that reduces one or more signs or symptoms can be identified by comparing the subjects treated with a control compound to the subjects treated
20 with the test compound. Techniques known to physicians familiar with weight disorders can be used to determine whether a candidate compound reduces one or more signs or symptoms associated with the weight disorder. For example, a candidate compound that enhances lipid metabolism will be beneficial for treating subjects having obesity.

In another embodiment, candidate compounds that reduce the blood glucose,
25 increase insulin sensitivity, increase insulin secretion, reduce the dose requirements of other anti-diabetic agents, or reduce the severity of one or more signs or symptoms associated with diabetes are identified in human subjects having diabetes. In accordance with this embodiment, a candidate compound or a control compound is administered to a human subject having diabetes, and the effect of a candidate compound on blood glucose, insulin
30 sensitivity, insulin secretion, dose requirements of other anti-diabetic agents, or one or more signs or symptoms of diabetes is determined. A candidate compound that reduces the blood glucose, increases insulin sensitivity, reduces the dose requirements of other anti-diabetic agents, or reduces one or more signs or symptoms associated with diabetes can be identified by comparing the subjects treated with a control compound to the subjects treated with the
35 test compound. Techniques known to physicians familiar with diabetes can be used to

determine whether a candidate compound reduces one or more signs or symptoms associated with diabetes.

In a preferred embodiment, a candidate compound that reduces the severity of one or more signs or symptoms associated with a weight disorder in a human having a weight disorder is selected for further testing or therapeutic use. In another preferred embodiment, 5 a candidate compound that reduces the severity of one or more signs or symptoms associated with diabetes in a human having a diabetes is selected for further testing or therapeutic use.

5.9. Diagnostic and Monitoring Techniques

10 In accordance with the present invention, test samples of adipose tissue, serum, or plasma obtained from a subject suspected of having or known to have a lipid metabolic disorder, a disorder characterized by lipid accumulation, or a weight disorder characterized by aberrant perilipin expression can be used for diagnosis or monitoring. In one embodiment, a decreased abundance of one or more perilipin isoforms (or any combination 15 of them) in a test sample relative to a control sample (from a subject or subjects free from a lipid metabolic disorder or a weight disorder) or a previously determined reference range indicates the presence of a lipid metabolic disorder or a weight disorder characterized by weight gain such as obesity. In another embodiment of the invention, an increased abundance of one or more perilipin isoforms (or any combination of them) in a test sample 20 compared to a control sample or a previously determined reference range indicates the presence of a weight disorder characterized by weight loss such as cachexia or anorexia. In yet another embodiment, the relative abundance of one or more perilipin isoforms (or any combination of them) in a test sample relative to a control sample or a previously determined reference range indicates the degree or severity of lipid metabolic disorder, a 25 disorder characterized by lipid accumulation, diabetes, or weight disorder (*e.g.*, obesity). In any of the aforesaid methods, detection of one or more perilipin isoforms described herein may optionally be combined with detection of one or more additional biomarkers for a lipid metabolic disorder or weight disorder such as, for example, leptin and neuropeptide Y. Any suitable method in the art can be employed to measure the level of a perilipin isoform, 30 including but not limited to immunoassays to detect and/or visualize a perilipin isoform (*e.g.*, Western blot, immunoprecipitation followed by sodium dodecyl sulfate polyacrylamide gel electrophoresis, immunocytochemistry, etc.). Further, any suitable hybridization assay can be used to detect perilipin isoform expression by detecting and/or visualizing mRNA encoding the perilipin isoform (*e.g.*, Northern assays, dot blots, *in situ* 35 hybridization, etc.).

In another embodiment of the invention, labeled antibodies, derivatives and analogs thereof, which specifically bind to a perilipin isoform can be used for diagnostic purposes to detect, diagnose, or monitor a lipid metabolic disorder or weight disorder characterized by aberrant perilipin expression. Preferably, such disorders are detected in animals, more preferably in mammals and most preferably in humans.

5

5.10. Kits

The present invention also provides for kits comprising one or more agents identified by the screening assays of the invention, and instructions for use. In one embodiment, a kit comprises one or more agonists of one or more perilipin isoforms, in one or more containers. In another embodiment, a kit comprises one or more antagonists of one or more perilipin isoforms, in one or more containers. Preferably, the kits of the present invention further comprise a control which does not agonize or antagonize the expression and/or activity of one or more perilipin isoforms.

In a specific embodiment, the kits of the present invention contain a labeled agonist or antagonist of one or more perilipin isoforms. In a preferred embodiment, the kits of the invention contain an agonist or antagonist of one or more perilipin isoforms conjugated to a therapeutic agent. In another preferred embodiment, the kits of the present invention contain a an agonist or antagonist of one or more perilipin isoforms conjugated to a diagnostic agent.

In certain embodiments, the kits of the invention contain instructions for the use of the antibodies for the treatment, prevention or diagnosis of a weight disorder (*e.g.*, obesity), a lipid metabolic disorder, a disorder characterized by lipid accumulation, or diabetes.

In order that the invention described herein may be more fully understood, the following examples are set forth. It should be understood that these examples is for illustrative purposes only and are not to be construed as limiting this invention in any manner.

6. **EXAMPLE: THE ABSENCE OF PERILIPIN PRODUCES GENETIC LEANNESS AND REVERSES THE OBESITY OF db/db MICE**

This example demonstrates the critical role that perilipin plays in lipid homeostasis, muscle mass and energy metabolism *in vivo*.

35

6.1. Materials & Methods

Production And Maintenance Of *plin*^{-/-} Mice

A mouse perilipin cDNA obtained by PCR was used to isolate a λ -phage clone from a mouse 129 genomic library. This clone contained exons 1-7 of the perilipin gene. A replacement vector was produced as shown in FIG. 1. The construct was introduced into
 5 mouse ES cells (R1, obtained from Dr. Andras Nagy of the University of Toronto) by electroporation (Chang et al., 1999, J. Biol. Chem. 274:6051-6055). Eight independent recombinant ES cell clones were injected into blastocysts derived from C57BL/6J. Genotyping was performed by tail blots using Xba I restriction enzyme. All experiments were performed in F3 and F4 mice backcrossed to C57BL/6J. The mice were weaned at
 10 4 weeks and were fed either a regular chow (standard Purina Rodent Chow containing 4.5% fat) or a high fat (HF) diet (F3282 from Bio-Serv, Frenchtown, NJ, containing 35% fat, 21% protein, and 38% carbohydrate).

Immunoblotting.

15 Equivalent amounts of protein homogenates were resolved by 4-15% SDS-PAGE, transferred to PVDF membrane and probed with polyclonal anti-hormone sensitive lipase (HSL) (anti-HSL antibodies were kindly provided by Dr. C. Holm, Lund University, Lund, (Sweden) and anti-perilipin antibodies (Research Diagnostics Inc., Flanders, NJ). Primary antibodies were visualized with enhanced chemiluminescence (ECL kit, Amersham
 20 Pharmacia Biotech). The relative intensity of the immunoblot bands was quantified by AlphaImager TM 2000 Documentation & Analysis System (Alpha Innotech Corp.).

Body Composition

Five *plin*^{+/+} and *plin*^{-/-} male mice were killed by cervical dislocation. Epididymal fat
 25 pad and gastrocnemius muscles were excised and weighted. The whole carcass was then homogenized in a blender. Fat was extracted with ethyl ether and ethyl alcohol from a preweighed portion of the ground carcass, so that percent fat could be calculated from the amount of material remaining after the extraction procedure. Carcass triacylglycerol (Sigma) and protein (Bio-Rad Protein Assay) were measured.

30

Glucose Tolerance And Insulin Sensitivity Tests

For glucose tolerance, mice were fasted for 4 hours and then injected intraperitoneally (i.p.) at dose of 3 g glucose per kg of body weight. Glucose levels were monitored before and after injection using blood glucose strips (FasTake, LifeScan Inc.,
 35 Milpitas, CA). For insulin sensitivity, mice were fasted for 4 hours and injected intraperitoneally with 100 units/ml of regular insulin, resulting in a final concentration of

0.75 U/kg body weight. Blood was collected before injection and at 15, 30, 60 and 120 minutes after injection. Glucose was measured using blood glucose strips.

Blood Chemistries

5 Blood was collected from the orbital plexus after animals were anesthetized with isoflurane (Vedco, St. Joseph, MO). Serum was frozen in aliquots and stored at -20°C. Enzymatic assay kits were used for the determination of serum nonesterified fatty acids (NEFA C, Wako, Richmond, VA), glycerol, glucose, cholesterol, β -hydroxybutyrate and total triacylglycerol (Sigma). Serum insulin was measured using radioimmunoassay (Linco
10 Research, St. Charles, MO). For glucose and insulin tolerance, glucose was measured by blood glucose strips (FasTake). Corticosterone (Diagnostic Products Co., Los Angeles, CA) and leptin concentration (Linco Research, St. Charles, MO) were determined by radioimmunoassay on plasma obtained at noon after an overnight fast.

Hormone-Sensitive Lipase Assays

15 Tissues were homogenized in 3 ml of buffer per gram of tissue (0.25 sucrose, 1 mM EDTA, 1 mM DTE, 20 μ g/ml leupeptin) and centrifuged at 110,000 x g for 45 minutes at 4°C. The fat-depleted infranant was used for measuring HSL activity essentially as described by Holm and coworkers (Holm et al., 1997, Methods Enzymol. 286:45-67; and
20 Osterlund et al., 1996, Biochem. J. 319:411-420) using the diolein analogue 1(3)-mono- ^{3} H]oleoyl-2-O-mono-oleylglycerol (MOME) as substrate. One unit of enzyme activity is defined as 1 μ mol of oleic acid released per minute 37°C and lipase activity was expressed as unit per mg of tissue.

Lipolysis In Isolated Adipocytes

25 Adipocytes were isolated from epididymal fat pads by collagenase digestion and in presence of adenosine as described (Rodbell, M., 1984, J. Biol. Chem. 239:375-380). Cells were resuspended in Kerbs-Ringer Hepes in absence of adenosine and in presence of 1 U/ml adenosine deaminase. 0.350×10^6 cells/ml cells were incubated for 1 hour in presence or
30 absence of 2 μ M CL 316,243 and extracellular glycerol release was measured as indicator of lipolysis.

Lipolysis In Vivo

Mice were fasted 4 hours and injected i.p. with CL 316,243 (0.1 mg per kg body
35 weight) or isoproterenol (10 mg per kg body weight). Blood was collected from the orbital plexus before and 15 minutes after injection and NEFA and glycerol were determined.

Histology

Tissues were fixed with neutral-buffered formalin and embedded in paraffin. Sections were stained with hematoxylin and eosin. Image was captured and analysis was performed with SigmaScan (Jandel, San Rafael, CA). The contour of each adipocyte was traced by hand the cytoplasmic area was determined. The size and distribution of brown
5 adipocytes could not be determined accurately because of the small lipid droplets obscuring the cellular boundaries *plin*^{-/-} mice. The average size was estimated by dividing the total surface area by the number of nuclei.

Oxygen Consumption Measurements

10 Oxygen consumption was assessed individually in mice fed a regular chow or a 35% fat diet using a computer-controlled open-circuit indirect calorimetry (Oxymax, Columbus Instruments Co., Columbus, OH) with an air flow of 0.51 min⁻¹ and a room temperature of 23°C. After 30 min allowed for the mice to adapt to the metabolic chamber. VO₂ was assessed at 5-min intervals for a 20- to 24-h period. Mice had free access to water and food
15 during the 12 hour night period. Total oxygen consumption represents the mean of all samples collected during the experiment.

Magnetic Resonance Imaging

Two sets of magnetic resonance imaging (MRI) experiments were performed. The
20 first set was to determine the 'fat' or 'lipid' image and the 'water' image. This was performed on *plin*^{-/-} and *plin*^{+/+} mice fixed in formalin. Preserved specimens were securely positioned inside a 25 mm NMR tube and imaged in a vertical bore, 9.4 Tesla MRI system (Varian, Palo Alto). Chemical shift selective saturation on either the water peak (4.8 ppm) or the lipid peak (1.2 ppm) was achieved using a series of three 4.0 ms sinc pulses, each
25 followed by a crusher gradient. A 25-slice spin echo imaging pulse sequence was used. Contiguous 1-mm slices were selected using 4 ms sinc pulses for excitation and refocussing. Each of the 256 phase encoder (PE) steps was acquired with a 4-sec repetition time, a 30 msec echo time and 32 signal averages per PE step. A quantitative assessment of the percent fat content in selected slices was made at the levels of the heart and liver. The
30 cross-sectional area of fat, determined from the fat image, was divided by the total cross-sectional area of mouse tissue in the slices, determined from the water image. Cross-sectional areas were quantified using image analysis software which segments the tissue according to signal intensity and counts the pixels.

In a second set of experiments, mice were anesthetized with Avertin and MRI
35 spectra representing water and lipid signals were determined in live *plin*^{+/+} and *plin*^{-/-} mice as described in the legend to FIG. 6D.

6.2. Results

Perilipin (*plin*) null mutant mice were produced by the targeted disruption of the perilipin gene, using the strategy shown in FIGS. 1A & 1B. The targeting vector replaced parts of exon 2 and intron 2 with an IRES- β -gal and the *neo* gene. Multiple targeted ES cell clones were obtained; of eight chimeras, five transmitted the targeted allele to the progeny. Homozygous and heterozygous mice were recovered in the expected proportions. Northern blotting revealed that perilipin mRNA was undetectable (data not shown), while Western blotting showed that perilipin protein was absent in the adipocytes (perilipin A and B) as well as testis (perilipin A and C) (FIG. 1C). *plin*^{-/-} mice had no overt abnormal phenotype; they were fertile and nursed their pups normally. When fed *ad libitum*, they consumed significantly more chow than *plin*^{+/+} littermate controls (0.429 ± 0.054 kcal/day/g body weight for *plin*^{-/-} and 0.332 ± 0.037 kcal/day/g body weight for *plin*^{+/+}, $p=0.011$). Despite their increased food intake, *plin*^{-/-} mice were not different from *plin*^{+/+} mice in their body weights (FIGS. 2A and 2B); the weights of their liver, kidney, spleen, and heart were also similar (data not shown). *plin*^{-/-} had less body fat than wild-type (*plin*^{+/+}) mice. Individual fat depots weighed 37-38% less in *plin*^{-/-} mice than *plin*^{+/+} mice (FIG. 2C). The knockout mice were, however, leaner than their *plin*^{+/+} mice, total carcass lipid content of *plin*^{-/-} mice was reduced by 58% (FIG. 2D) and triacylglycerol content reduced by 46% (FIG. 2E), while the total protein content of *plin*^{-/-} mice was actually increased by 21% (FIG. 2F). There was good correlation between the epididymal fat pad weight and total body triacylglycerol content ($r=0.920$, $p<0.001$), but not between body weight and fat pad weight ($r=0.199$, $p=0.582$) or triacylglycerol content ($r=0.052$, $p<0.887$). Not only were *plin*^{-/-} mice not cachectic, they actually had an increase in muscle mass, which allowed the mice to maintain normal body weight despite the loss of body fat. The weight of gastrocnemius muscle in *plin*^{-/-} mice was 7.8% higher than that of *plin*^{+/+} controls ($p<0.05$, $n=9$ for both groups, FIG. 2G).

Adipocytes from *plin*^{-/-} mice were substantially smaller than those from *plin*^{+/+} littermates (FIG. 3A). The average white adipocyte size (area) in histological sections (FIG. 3B) in *plin*^{+/+} mice was 3757 ± 1272 μm^2 , while that in *plin*^{-/-} mice was 1400 ± 48 μm^2 , a 62% reduction in size. The much smaller white adipose depots in *plin*^{-/-} mice were the result of a diminution in size of individual fat cells, not by a reduction in fat cell number, because the cell density was much higher in *plin*^{-/-} than in *plin*^{+/+} mice (FIG. 3C), and the total DNA content of the adipose tissue was similar in *plin*^{-/-} and *plin*^{+/+} animals (152 ± 56 μg in *plin*^{-/-} vs 140 ± 85 μg in *plin*^{+/+}, $n=6$ in both groups). The interscapular brown fat was smaller in *plin*^{-/-} than in *plin*^{+/+} mice (FIG. 3D). Although the mean size of fat cells in the interscapular brown adipose tissue tended to be smaller, the difference was not significant (2.607 ± 0.209 μm^2 in *plin*^{-/-} compared with 3.468 ± 1.018 μm^2 in *plin*^{+/+}, $p=0.071$).

However, the histological appearance was very different, because the lipid droplets in *plin*^{-/-} brown adipocytes were much smaller than those in *plin*^{+/+} brown adipocytes (FIG. 3A). The decrease in total body fat in the *plin*^{-/-} mice was reflected by a corresponding reduction in their plasma leptin concentration (for male mice, 2.26 ± 1.23 ng/ml in *plin*^{-/-}, n=10, vs 3.64 ± 1.40 ng/ml in *plin*^{+/+}, n=7, p=0.056; and for female mice 1.78 ± 0.58 ng/ml in *plin*^{-/-}, n=10, vs 3.02 ± 1.40 ng/ml in *plin*^{+/+}, n=8, p=0.001). There was no abnormal liver histology and no evidence of fatty infiltration in the liver in the *plin*^{-/-} mice (data not shown).

Despite a major reduction in total body fat in the *plin*^{-/-} mice, no significant difference between *plin*^{-/-} and *plin*^{+/+} animals in the following parameters was detected: basal plasma cholesterol (*plin*^{+/+} 89.7 ± 11.4 mg/dl vs *plin*^{-/-} 97 ± 18.1 mg/dl) and triacylglycerol levels (*plin*^{+/+} 35.6 ± 6.8 mg/dl vs *plin*^{-/-} 29.1 ± 6.4 mg/dl), plasma lipoprotein profile analyzed by fast protein liquid chromatography (data not shown; Clifford et al., 2000, J. Biol. Chem. 273:24665-24669), fasting plasma glucose (*plin*^{+/+} 81.95 ± 20.03 mg/dl vs *plin*^{-/-} 81.95 ± 11.24 mg/dl) and insulin (*plin*^{+/+} 0.179 ± 0.081 ng/ml vs *plin*^{-/-} 0.206 ± 0.096 ng/ml), plasma glucose response over a 2 hour period to an insulin tolerance test using a standard intraperitoneal (i.p.) insulin dose of 0.75 U/kg (data not shown), and plasma glucose and insulin response over a 2 hour period to a standard glucose tolerance test using 3 g/kg glucose administered i.p. (data not shown). There was also no difference in the plasma corticosterone levels between *plin*^{-/-} (118.28 ± 54.45 ng/ml for males, n=10, and 228.92 ± 56.28 ng/ml for females, n=9) and *plin*^{+/+} (108.49 ± 62.25 ng/ml for males, n=10, and 188.55 ± 86.31 ng/ml for females, n=10).

The effect of a 48 hour fast on some plasma parameters is shown in FIG. 4A. There was no difference in any of the parameters at the beginning of the fast. The major difference in response to the fast was in the level of the lipolysis metabolite, nonesterified fatty acids (NEFA), and the ketone body, β -hydroxybutyrate. Wild-type mice were able to increase their plasma NEFA and β -hydroxybutyrate following the fast. In contrast, if *plin*^{-/-} mice were able to mount a lipolytic response, it was not sufficient to elevate the level of NEFA. The increase in plasma β -hydroxybutyrate after the fast was also substantially attenuated (FIG. 4A). Most likely in *plin*^{-/-} mice the much smaller fat depot, which was already maximally stimulated under basal conditions, failed to increase its release of NEFA (see below). In turn, the production of ketone bodies from acetyl CoA via the β -oxidation of fatty acids was impaired because the supply of NEFA was limiting.

Perilipin has been reported to modulate hormone-sensitive lipase (HSL) activity; its absence might affect HSL activation, thereby changing the rate of lipolysis and energy balance of at cells (Londos et al., 1999, Semin. Cell Dev. Biol. 19: 51-58). By Western blotting (FIG. 4B), no significant difference in immunoreactive HSL between *plin*^{+/+} and *plin*^{-/-} mice was detected. HSL activity in cell lysates from *plin*^{-/-} mice in subcutaneous fat

and in epididymal fat was 287% and 652%, respectively, of the corresponding fat depots in *plin*^{+/+} controls (FIG. 4C). The constitutively activated HSL in *plin*^{-/-} mice indicates that perilipin normally functions by reining in adipose HSL activity. The reduced HSL activity in the presence of a normal amount of the protein indicates the perilipin regulates HSL activity, perhaps by affecting its access to triacylglycerol in the fat droplets (Londos et al., 1999, Semin. Cell Dev. Biol. 19: 51-58), and not its overall production.

Total lipolytic activity of isolated adipocytes was measured next. Basal glycerol release from isolated fat cells of *plin*^{-/-} mice was approximately 375% of that from adipocytes of *plin*^{+/+} mice (FIG. 4D). Addition of CL 316,243 (2 μ M), a specific agonist for β -adrenergic receptor that is predominantly expressed in adipose tissues in rodents (Muzzin et al., 1991, J. Biol. Chem. 266:24053-24058), stimulated glycerol release about 300% in *plin*^{+/+} adipocytes, to a value approximating that in untreated *plin*^{-/-} adipocytes. In contrast, in adipocytes from *plin*^{-/-} mice, lipolytic activity was already maximal under basal conditions and CL 316,243 did not produce any further stimulation.

These *in vitro* observations prompted the examination of the effect of perilipin inactivation on lipid metabolism *in vivo*. The rate of lipolysis in *plin*^{-/-} and *plin*^{+/+} mice was studied by measuring the products of lipolysis, glycerol and NEFA, under basal conditions and following the administration of isoproterenol (10 mg/kg IP), a general β -adrenergic agonist, and CL 316,243 (0.1 mg/kg IP). Blood was collected before and 15 minutes after i.p. administration of either compound at a time coincident with the maximal plasma glycerol and NEFA response (FIG. 4E). Before treatment, despite the much lower body fat content of *plin*^{-/-} mice, basal plasma glycerol and NEFA levels were approximately similar in *plin*^{-/-} and *plin*^{+/+} mice. After isoproterenol treatment, there was an approximately 800% increase in the level of plasma glycerol, and an approximately 300% increase in the level of plasma NEFA in *plin*^{+/+} mice. The corresponding increase was much attenuated in *plin*^{-/-} mice, in which glycerol and NEFA levels went up approximately 300% and 0%, respectively. A similar observation was evident with CL 316,243 treatment. The basal glycerol and NEFA levels were again very similar in these experiments. In *plin*^{+/+} mice, glycerol and NEFA levels were stimulated approximately 1100% and approximately 420%, respectively, by CL treatment. In *plin*^{-/-} mice, the stimulation by CL 316,243 was much less, amounting to approximately 200% and approximately 140%, respectively (FIG. 4E). The difference in basal and stimulated levels can be easily explained by taking into consideration the greatly reduced adipose tissue mass in *plin*^{-/-} mice compared with *plin*^{+/+} controls. The *in vitro* lipolysis experiments (FIG. 4D) indicate that *plin*^{-/-} adipocytes exhibit near-maximal lipolysis under basal conditions. Thus, despite a markedly reduced adipose mass, *plin*^{-/-} mice were able to maintain relatively normal basal plasma glycerol and NEFA levels.

Furthermore, the *in vivo* experiments confirmed what was observed *in vitro*; *i.e.*, *plin*^{-/-} adipocytes are poorly stimulated by exposure to β -adrenergic agonists.

At an ambient temperature of 21 °C, *plin*^{-/-} and *plin*^{+/+} mice had similar body temperatures (FIG. 5A). It is possible that *plin*^{-/-} mice might be poorly insulated from changes in environmental temperature because of their markedly reduced subcutaneous fat. Thus, the ability of *plin*^{-/-} mice to maintain their body temperature on exposure to cold was examined. When the mice were subjected to an ambient temperature of 4 °C in the fasted state (FIG. 5B), the body temperature of the *plin*^{-/-} mice fell much faster than that of their *plin*^{+/+} littermates, an observation consistent with poor heat conservation. However, when the experiment was repeated in the fed state (FIG. 5C), *plin*^{-/-} animals were able to withstand the cold as well as their *plin*^{+/+} littermates (for at least 9 hours), indicating that they had overcome their poor heat conservation with a thermogenic response. In fact, in the fed state, the body temperature of *plin*^{-/-} mice tended to be slightly higher than that of *plin*^{+/+} mice, indicating that the metabolism of food (and oxygen) generated enough heat to keep the body temperature at a high normal range, despite increased heat loss from poor insulation.

To document whether *plin*^{-/-} mice had an increased metabolic rate, the total oxygen consumption of *plin*^{-/-} and *plin*^{+/+} mice by direct calorimetry was examined. *plin*^{-/-} mice consumed slightly (approximately 11%) more O₂ than wild-type animals when they were fed regular chow (47.6 ± 3.6 ml/kg/min in *plin*^{-/-} vs 42.8 ± 3.0 ml/kg/min in *plin*^{+/+} 11-week-old mice, n=5; p=0.052; on a per animal basis, they were 1.250 ± 0.179 in *plin*^{-/-} vs 1.161 ± 0.074 ml/min in *plin*^{+/+}; p=0.334); the difference was substantially greater (20%) when they were on a high-fat diet (59.2 ± 3.2 ml/kg/min, n=4, in *plin*^{-/-} vs 50.0 ± 1.9 ml/kg/min, n=6, in *plin*^{+/+} 11-week old mice; p<0.001; on a per animal basis, they were 1.486 ± 0.077 ml/min for *plin*^{-/-} vs 1.340 ± 0.088 ml/min for *plin*^{+/+}, p=0.024). The difference in O₂ consumption between *plin*^{-/-} and *plin*^{+/+} mice was greater when the mice were awake and eating than when they were sleeping (FIG. 5D).

After eating a high (35%) fat diet for 3.5 months, *plin*^{+/+} mice became obese, whereas *plin*^{-/-} mice were relatively resistant to the diet-induced obesity (FIG. 6A and 6B). Under these conditions wild-type mice developed huge epididymal fat pads. Although the epididymal fat pads of *plin*^{-/-} mice also increased in size, they attained a relative mass that was not different from that observed in wild-type animals on a regular chow diet (FIG. 6A). Furthermore, after the high-fat diet feeding, the total body (carcass) fat content was almost 50% lower in *plin*^{-/-} mice compared with *plin*^{+/+} mice (21.7% in *plin*^{-/-} and 41.4% in *plin*^{+/+} mice FIG. 6B).

Inactivation of the perilipin gene also protected *db/db* mice, a genetic model of obesity caused by leptin resistance (Chen et al., 1996, Cell 84:491-495), from developing obesity. By intercrossing the two types of animals, *db/db* mice that inherited the *plin*^{-/-}

alleles were obtained; remarkably, these mice had lost the obesity phenotype (FIG. 6C and 6D). The protective effect of the absence of perilipin, was evident early in life. At 6 weeks, wild-type mice weighed 17.59 ± 2.59 g (n=11), *db/db/plin^{+/+}* weighed 33.45 ± 1.61 g (n=4), and *db/db/plin^{-/-}* weighed 24.90 ± 2.16 g (n=3). The marked difference in weight between *db/db/plin^{-/-}* and *db/db/plin^{+/+}* persisted as the mice grew older. At 12 weeks, wild-type (n=11) and *db/db/plin^{+/+}* (n=5) weighed 24.93 ± 1.37 g and 49.83 ± 4.14 g, respectively, compared with a *db/db/plin^{-/-}* mouse that weighed 30.2 g. At 20 weeks, the weights were 28.78 ± 2.14 g for wild-type (n=11), 60.75 ± 2.09 g for *db/db/plin^{+/+}* (n=5), and 33.5 g for the *db/db/plin^{-/-}* mouse. As shown in FIGS. 7A and 7B, the body weight of *db/db/plin^{-/-}* mice approaches the body weight of wild-type mice as the *db/db/plin^{-/-}* mice age. Thus, the knockout of the perilipin alleles in *db/db* mice reverses, at least in part, the obesity phenotype associated with the of *db/db* genotype. Further, *db/db/plin^{-/-}* mice exhibited a delayed onset of diabetes relative to *db/db/plin^{+/+}* mice.

Magnetic resonance imaging (MRI) was used to quantify areas of fat in sections of *plin^{-/-}* mouse and *plin^{+/+}* control. Fat image represented approximately 7.6% of the total cross-sectional area in a slice through the heart region of *plin^{-/-}*, compared to approximately 15% in *plin^{+/+}* animal. In a slice in the region of the liver, the fat area was approximately 3.5% in *plin^{-/-}* compared to approximately 9.24% in *plin^{+/+}*. Thus, the reduction in fat area in individual sections was 50-65% (data not shown). However, when the whole-body MRI was used, the total lipid content of *plin^{-/-}* (FIG. 6D, top right panel) was reduced approximately 36% compared with *plin^{+/+}* mice (FIG. 6D, top left panel). Similar analyses of *db/db/plin^{+/+}* and *db/db/plin^{-/-}* mice revealed that the subcutaneous and visceral fat in *db/db* was markedly diminished, with the total lipid signal decreasing from approximately 63% in *db/db/plin^{+/+}* to approximately 27% in *db/db/plin^{-/-}*, a value approximating that in wild-type (approximately 24%) (FIG. 6D, bottom panels). These MRI analyses confirm that inactivation of the perilipin in mice reverses the obesity phenotype of *db/db* mice. The loss of the excess body fat in the double knockout mice was associated with an increase in metabolic rate as assessed by oxygen consumption. The difference in VO_2 was evident whether the measurements were taken when the mice were eating or sleeping (FIG. 5E).

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6.3. Discussion

Applicant has generated *plin^{-/-}* mice, which are healthy, muscular, and lean. These mice are resistant to diet-induced obesity and the inheritance of the *plin^{-/-}* alleles in *db/db* mice reverses their obesity phenotype. The obesity-resistance phenotype in the *plin^{-/-}* mice is a result of their high metabolic rate. One mechanism for the increased energy expenditure in these mice is their greater lean body mass, which is metabolically active. Another

possible mechanism is that the free fatty acids produced in the fat cells may be reesterified *in situ*, and there is a futile cycle of lipogenesis and lipolysis that is consuming ATP and leading to increased oxygen consumption.

plin^{-/-} mice are very different from the recently described obesity-resistant Hmgic-deficient mice, which have a pygmy phenotype (Zhou et al., 1995, Nature 376:771-774; Anand et al., 2000, Nat. Genet. 24:377-380; Hirning-Folz et al., 1998, Genes Chrom. Cancer 23:350-357; and Benson et al., 1994, Genet. Res. 64:27-33). Adipocytes are one of many mesenchymal tissues affected by Hmgic deficiency. Unlike *Hmgic*^{-/-} mice, which are >50% lighter than wild-type littermates (Anand et al., 2000, Nat. Genet. 24:377-380), *plin*^{-/-} mice have a normal body weight and increased muscle mass despite a reduced adipose content.

plin^{-/-} mice show some superficial similarity to mice with inactivated acyl CoA:diacylglycerol transferase (*Dgat*; Smith et al., 2000, Nature Genet. 25:87-90) and those with inactivated protein kinase A RII β subunit (Cummings et al., 1996, Nature 382:622-626). All three types of mice display increased energy expenditure and constitutional leanness (Smith et al., 2000, Nature Genet. 25:87-90; and Cummings et al., 1996, Nature 382:622-626). The mechanism for the phenotypic manifestations of *Dgat* inactivation, which include failure of normal milk production, is unclear (Smith et al., 2000, Nature Genet. 25:87-90). Inactivation of protein kinase A RII β subunit stimulates lipolysis apparently as a result of a compensatory increase in protein kinase A RI α subunit (Cummings et al., 1996, Nature 382:622-626). Protein kinase A RII β subunit is expressed in adipose tissue and brain, as well as at low levels elsewhere. One possible interpretation of the data from mice with inactivated protein kinase A RII β subunit is that the lean phenotype of RII β inactivation "derives more directly from neuronal alterations in protein kinase A" activity than from adipose RII β inactivation (Planas et al., 1999, J. Biol. Chem. 274:36281-36287). Perilipin, in contrast, is expressed exclusively in adipose tissue, and at very low levels in steroidogenic tissues. Its inactivation within adipocytes appears to be directly responsible for the increased lipolytic activity, raised basal metabolic rate, and the constitutional leanness.

7. **EXAMPLE: THE ABSENCE OF PERILIPIN REVERSES THE OBESITY OF *ob/ob* MICE**

This example demonstrates the critical role that perilipin plays in lipid and energy metabolism *in vivo*. This example also demonstrates the beneficial effects that result from the downregulation of perilipin expression or activity.

7.1. **Materials & Methods**

***ob/ob/plin*^{-/-} Mice**

db/db/plin^{-/-} mice were generated by intercrossing *ob/ob* mice and *plin*^{-/-} mice.

Northern Blot Analysis:

RNA was isolated from the various tissues by Ultraspec RNA isolation kit from Biotecz, Houston, Texas. Hybridization probes used were the corresponding ³²P-labeled cloned mouse cDNAs. The RNA (20 µg) was electrophoresed on 1% agarose gele and transferred to Hybond-N nylon membrane filters. The filters were hybridized to the corresponding ³²P-labeled cDNA probes (see FIGS. 10-12) at 42°C for 16 hours and then washed sequentially with 2 X SSC, 0.05% SDS, and 2 X SSC, 0.1% SDS, and finally with 0.1 X SSC, 0.1% SDS at 65°C for 30 minutes (1 X SSC = 15 mM sodium citrate, 150 mM sodium chloride, pH 9.0). The filters were exposed to X-ray films with two intensifying screens at -80°C for 24-72 hours.

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Glucose Tolerance Test

For glucose tolerance, mice were fasted for 10 hours and then injected intraperitoneally (i.p.) at dose of 1.5 g glucose per kg of body weight. Glucose levels were monitored before and after injection using blood glucose strips (FasTake, LifeScan Inc., Milpitas, CA). Insulin levels were monitored before and after injection using an ELISA kit from Crystal Chem Inc. (Chicago, ILL).

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7.2. Results

Inactivation of the perilipin gene protected *ob/ob* mice, a genetic model of obesity, from developing obesity. The protective effect of the absence of perilipin, was evident early in life. As shown in FIGS. 8A and 8B, the body weight of *ob/ob/plin*^{-/-} mice was reduced relative to the body weight of *ob/ob/plin*^{+/+} mice. The body weight of *ob/ob/plin*^{-/-} mice approaches the body weight of wild-type mice as the *ob/ob/plin*^{-/-} mice age. Thus, the knockout of the perilipin alleles in *ob/ob* mice reverses, at least in part, the obesity phenotype associated with the of *ob/ob* genotype.

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Next, Northern blot assays were performed to determine the effect of perilipin inactivation on the expression of enzymes involved in β-oxidation. FIG. 9 depicts the steps in β-oxidation. As shown in FIGS. 10 and 11, increased mRNA levels for the major β-oxidation enzymes were detected mainly in adipose tissue, the heart, and muscle. These results suggest that one mechanism by which perilipin inactivation leads to the breakdown of fat is through activated β-oxidation. One working hypothesis is that the synthesis of triacylglycerol is either unchanged or only secondarily changed in a minor degree such that any triacylglycerol synthesized is immediately degraded by lipolysis in the *plin*^{-/-} mice, creating a futile cycle that wastes ATP.

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Since UCPs are known to reduce efficiency of energy consumption by “wasting ATP” and generating thermal energy, the effect of perilipin inactivation on the stimulation

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of uncoupled protein (UCP) activity was assessed. There are three closely homologous UCPs, UCP-1, UCP-2, and UCP-3, and a few other substantially less homologous ones. As shown in FIG. 12, there was no difference in the concentration of UCP-1 mRNA expression in brown adipose tissue, but there was an increase in UCP-2 mRNA concentration detected in brown and white adipose tissue and the heart using Northern blot analysis. These results suggest that the induction of UCPs is one possible mechanism that the *plin*^{-/-} mice have for increased metabolic rate and oxygen consumption.

The effect of perilipin inactivation on the glucose intolerance (diabetes mellitus) in *ob/ob* mice was assessed. Glucose and insulin concentrations from the plasma of wild-type and *ob/ob* mice with or without perilipin alleles were measured after a 10 hour fast. FIGS. 13A and 13B graphically depict the concentration of fasting plasma glucose and fasting plasma insulin, respectively, detected in mice. As shown in FIG. 14A, the blood glucose levels after i.p. administration of glucose to *ob/ob* mice revealed a diabetic curve. In contrast, the blood glucose levels in *ob/ob/plin*^{-/-} mice were not significantly different, if not indistinguishable, from wild-type controls. As shown in FIG. 14B, the blood insulin levels after i.p. administration of glucose to *ob/ob* mice were elevated relative to wild-type controls. The inactivation of the perilipin alleles reduced the level of blood insulin levels in *ob/ob* mice 30 minutes following the administration of glucose i.p. (FIGS. 14A and 14B). These results suggest that perilipin inactivation reverses glucose intolerance and reverses insulin secretion abnormality in *ob/ob* mice. There are two mechanisms for the beneficial effects of perilipin downregulation: (i) it decreases insulin resistance in *ob/ob* mice by reducing the amount of body fat; and (ii) it improves insulin secretion.

The present invention is not to be limited in scope by the exemplified embodiments, which are intended as illustrations of single aspects of the invention. Indeed, various modifications of the invention in addition to those shown and described herein will become apparent to those skilled in the art from the foregoing description and accompanying drawings. Such modifications are intended to fall within the scope of the appended claims.

All publications cited herein are incorporated by reference in their entirety.

WO 02/28410

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SEQUENCE LISTING

<110> Baylor College Of Medicine

<120> PERILIPIN AS A TARGET FOR REGULATION OF BODY WEIGHT, MUSCLE MASS AND DIABETES

<130> 9142-021-228

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WHAT IS CLAIMED IS:

1. A method of identifying an agent to be tested for an ability to modulate body weight, body fat or muscle mass, said method comprising:
 - 5 (a) contacting a perilipin isoform or a fragment thereof with a candidate agent for a time sufficient to form perilipin isoform or fragment/agent complexes; and
 - (b) measuring perilipin isoform or fragment/agent complex level, so that if the level measured differs from that measured in the absence of the candidate agent, then an agent to be tested for an ability to modulate body weight, body fat or muscle
10 mass is identified.
2. A method of identifying an agent to be tested for an ability to modulate the onset, development or progression of diabetes, said method comprising:
 - 15 (a) contacting a perilipin isoform or a fragment thereof with a candidate agent for a time sufficient to form perilipin isoform or fragment/agent complexes; and
 - (b) measuring perilipin isoform or fragment/agent complex level, so that if the level measured differs from that measured in the absence of the candidate agent, then an agent to be tested for an ability to modulate the onset, development or
20 progression of diabetes is identified.
3. A method of identifying an agent to be tested for an ability to modulate lipid metabolism, said method comprising:
 - 25 (a) contacting a perilipin isoform or a fragment thereof with a candidate agent for a time sufficient to form perilipin isoform or fragment/agent complexes; and
 - (b) measuring perilipin isoform or fragment/agent complex level, so that if the level measured differs from that measured in the absence of the candidate agent, then an agent to be tested for an ability to modulate lipid metabolism is identified.
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4. A method of identifying an agent to be tested for an ability to modulate body weight, body fat or muscle mass, said method comprising:
 - 35 (a) contacting a population of cells expressing a perilipin isoform with a candidate agent for a time sufficient to form perilipin isoform/agent complexes; and
 - (b) measuring perilipin isoform/agent complex level,

so that if the level measured differs from that measured in the absence of the candidate agent, then an agent to be tested for an ability to modulate body weight, body fat or muscle mass is identified.

5. A method of identifying an agent to be tested for an ability to modulate the onset, development or progression of diabetes, said method comprising:

- (a) contacting a population of cells expressing a perilipin isoform with a candidate agent for a time sufficient to form perilipin isoform/agent complexes; and
 - (b) measuring perilipin isoform/agent complex level,
- 10 so that if the level measured differs from that measured in the absence of the candidate agent, then an agent to be tested for an ability to modulate the onset, development or progression of diabetes is identified.

6. A method of identifying an agent to be tested for an ability to modulate lipid metabolism said method comprising:

- (a) contacting a population of cells expressing a perilipin isoform with a candidate agent for a time sufficient to form perilipin isoform/agent complexes; and
 - (b) measuring perilipin isoform/agent complex level,
- 20 so that if the level measured differs from that measured in the absence of the candidate agent, then an agent to be tested for an ability to modulate lipid metabolism is identified.

7. A method of identifying an agent to be tested for an ability to modulate body weight, body fat or muscle mass, said method comprising:

- (a) contacting a population of cells expressing a perilipin isoform with a candidate agent; and
- (b) measuring the level of induction of a cellular second messenger or the level of phosphorylation of the perilipin isoform,

so that if the level measured differs from that measured in the absence of the candidate agent, then a compound to be tested for an ability to modulate body weight, body fat or muscle mass is identified.

8. A method of identifying an agent to be tested for an ability to modulate the onset, development or progression of diabetes, said method comprising:

- (a) contacting a population of cells expressing a perilipin isoform with a candidate agent; and

- (b) measuring the level of induction of a cellular second messenger or the level of phosphorylation of the perilipin isoform,

so that if the level measured differs from that measured in the absence of the candidate agent, then a compound to be tested for an ability to modulate the onset, development or progression of diabetes is identified.

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9. A method of identifying an agent to be tested for an ability to modulate lipid metabolism, said method comprising:

- (a) contacting a population of cells expressing a perilipin isoform with a candidate agent; and

- 10 (b) measuring the level of induction of a cellular second messenger or the phosphorylation of the perilipin isoform,

so that if the level measured differs from that measured in the absence of the candidate agent, then a compound to be tested for an ability to modulate lipid metabolism is identified.

- 15 10. The method of claim 1, 4 or 7, wherein said ability to modulate body weight is the ability to decrease weight.

11. The method of claim 1, 4 or 7, wherein said ability to modulate body weight is the ability to increase weight.

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12. The method of claim 1, 4 or 7, wherein said ability to modulate body fat is the ability to decrease body fat.

13. The method of claim 2, 5 or 8, wherein said ability to modulate the onset,
25 development or progression of diabetes is the ability to reduce blood glucose levels, reduce insulin sensitivity, or reduce one or more signs or symptoms associated with diabetes.

14. The method of claim 1, 4 or 7, wherein said ability to modulate the muscle mass is the ability to increase muscle mass.

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15. The method of claim 3, 6 or 9, wherein said ability to modulate lipid metabolism is the ability to decrease lipid metabolism.

16. The method of claim 3, 6 or 9, wherein said ability to modulate lipid
35 metabolism is the ability to increase lipid metabolism.

17. A method for identifying an agent that modulates the body weight or body fat of an animal, said method comprising:

- (a) administering to an animal or group of animals a candidate agent that binds to one or more perilipin isoforms, modulates the expression of one or more perilipin isoforms, or modulates one or more activities of one or more perilipin isoforms; and
- (b) determining whether the candidate agent modulates the body weight or body fat in the animal or animals relative to an untreated control animal or animals,

so that if the candidate agent modulates the body weight or body fat, then an agent that modulates the body weight or body fat of an animal is identified.

18. A method for identifying an agent that modulates muscle mass in an animal, said method comprising:

- (a) administering to an animal or group of animals a candidate agent that binds to one or more perilipin isoforms, modulates the expression of one or more perilipin isoforms, or modulates one or more activities of one or more perilipin isoforms; and
- (b) determining whether the candidate agent modulates muscle mass in the animal or animals relative to an untreated control animal or animals,

so that if the candidate agent modulates the muscle mass, then an agent that modulates muscle mass in an animal is identified.

19. A method for identifying an agent that modulates the onset, development or progression of diabetes in an animal, said method comprising:

- (a) administering to an animal or group of animals a candidate agent that binds to one or more perilipin isoforms, modulates the expression of one or more perilipin isoforms, or modulates one or more activities of one or more perilipin isoforms; and
- (b) determining whether the candidate agent modulates blood glucose levels, insulin sensitivity, or one or more signs or symptoms of diabetes in the animal or animals relative to an untreated animal or animals,

so that if the candidate agent modulates blood glucose levels, insulin sensitivity or one or more signs or symptoms of diabetes, then an agent that modulates the onset, development or progression of diabetes in an animal is identified.

20. A method for identifying an agent that modulates lipid metabolism in an animal, said method comprising:

- 5 (a) administering to an animal or group of animals a candidate agent that binds to one or more perilipin isoforms, modulates the expression of one or more perilipin isoforms, or modulates one or more activities of one or more perilipin isoforms; and
- (b) determining whether the candidate agent modulates lipid metabolism in the animal or animals relative to an untreated control animal or animals,

so that if the candidate agent modulates the lipid metabolism, then an agent that modulates lipid metabolism in an animal is identified.

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21. The method of claim 17, 18, 19, or 20, wherein the animal or animals are livestock, poultry or companion animals.

15 22. The method of claim 17, 18, 19, or 20, wherein the animal or animals are human.

23. A method of preventing or treating a disorder characterized by weight gain in a subject, said method comprising administering to said subject in which such treatment is needed or desired a therapeutically effective amount of one or more compounds that

20 function as antagonists of perilipin expression or activity.

24. A method of preventing or treating a disorder characterized by weight loss in a subject, said method comprising administering to said subject in which such treatment is needed or desired a therapeutically effective amount of one or more compounds that

25 function as an agonists of perilipin expression or activity.

25. A method of preventing or treating a diabetes in a subject, said method comprising administering to said subject in which such treatment is needed or desired a therapeutically effective amount of one or more compounds that function as an antagonist of

30 perilipin expression or activity.

26. The method of claim 23, wherein the weight disorder is obesity.

35 27. The method of claim 24, wherein the disorder is cachexia or anorexia.

28. A method of enhancing lipid metabolism and increasing muscle mass in a subject, said method comprising administering to said subject in which such treatment is needed or desired a therapeutically effective amount of one or more compounds that function as antagonists of perilipin expression or activity.

5 29. A method of enhancing lipid accumulation in a subject, said method comprising administering to said subject in which such treatment is needed or desired a therapeutically effective amount of one or more compounds that function as agonists of perilipin expression or activity.

10 30. The method of claim 23, 24, 25, 27 or 28 in which the subject is a human.

31. The method of claim 23, 24, 25, 27 or 28 in which the subject is livestock or poultry.

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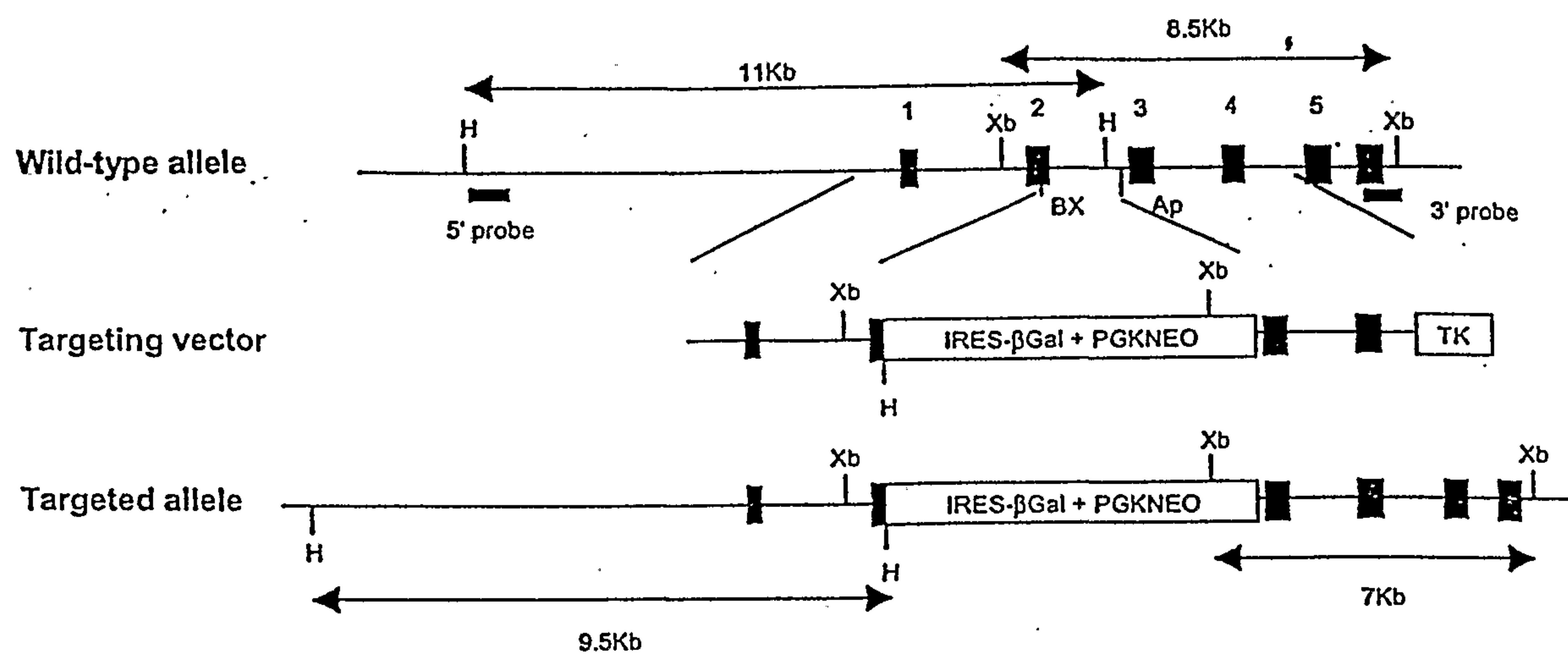


FIG. 1A

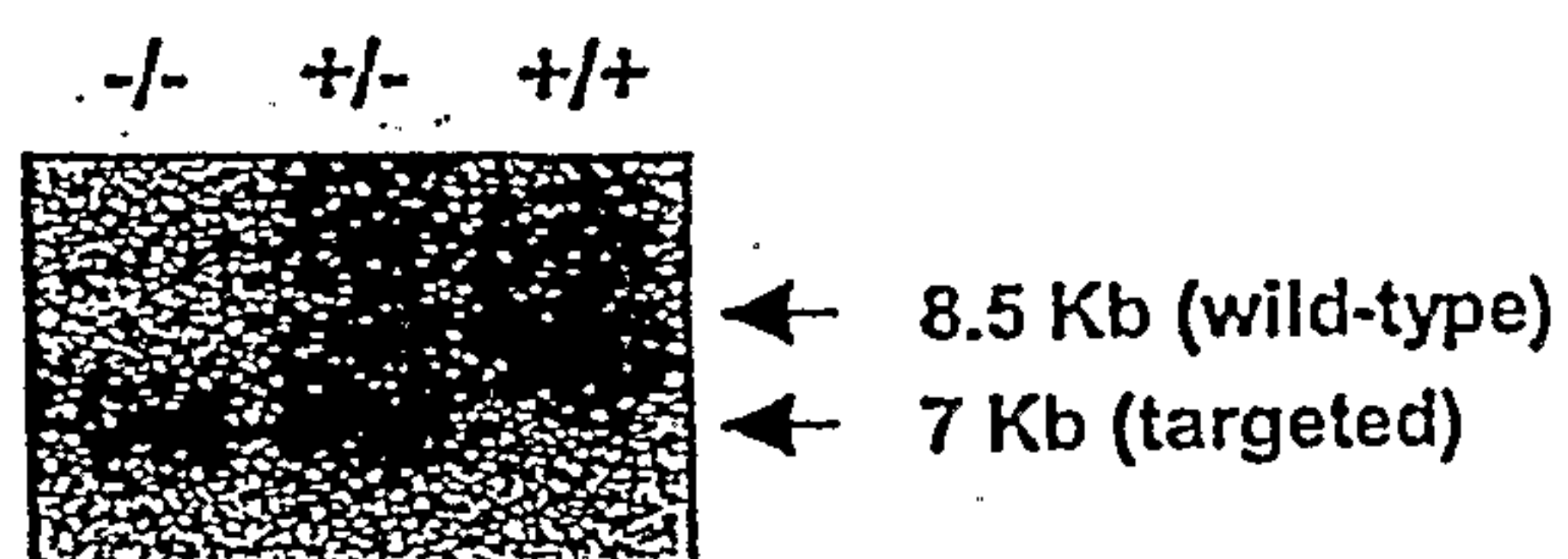


FIG. 1B

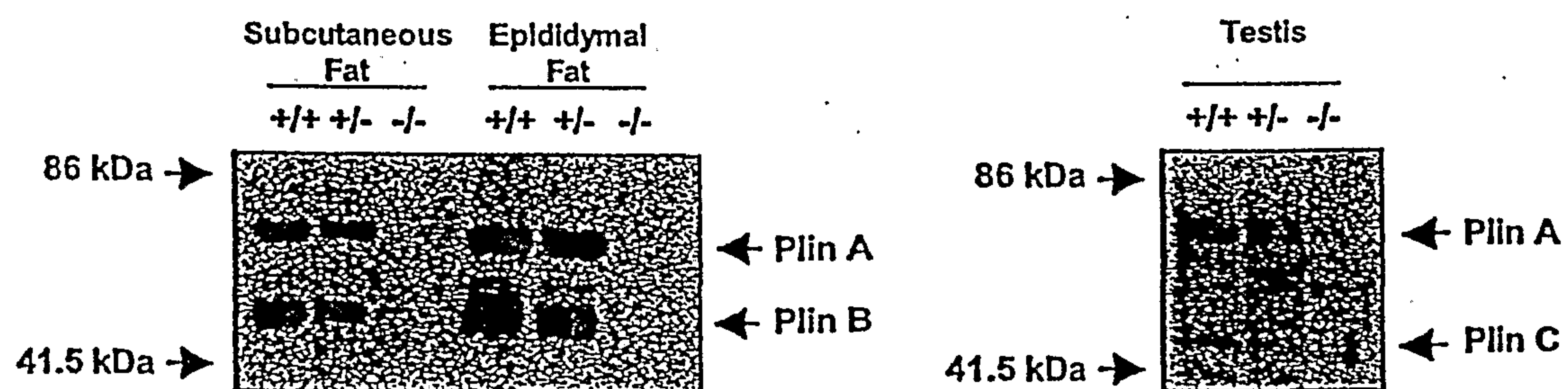


FIG. 1C

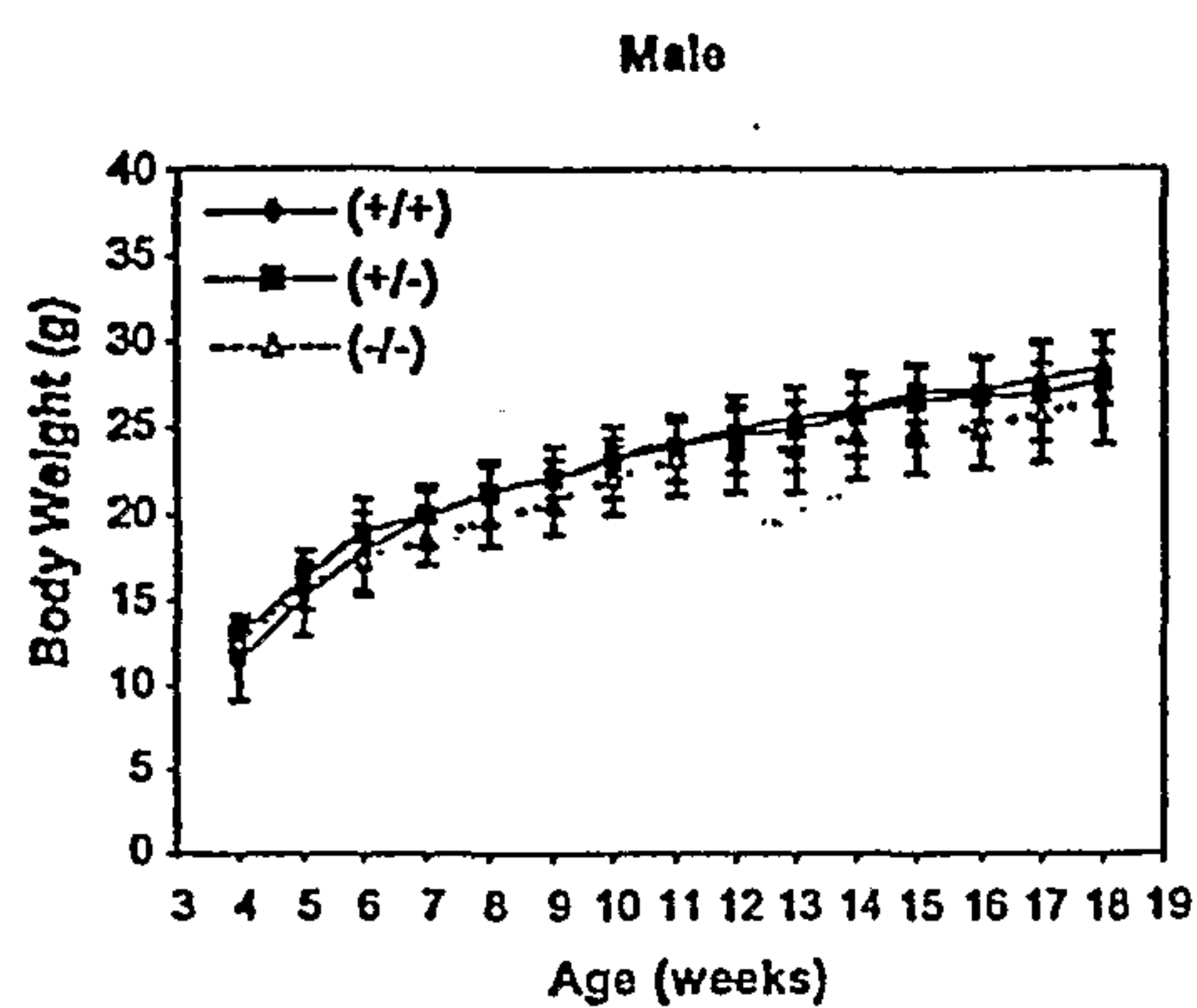


FIG. 2A

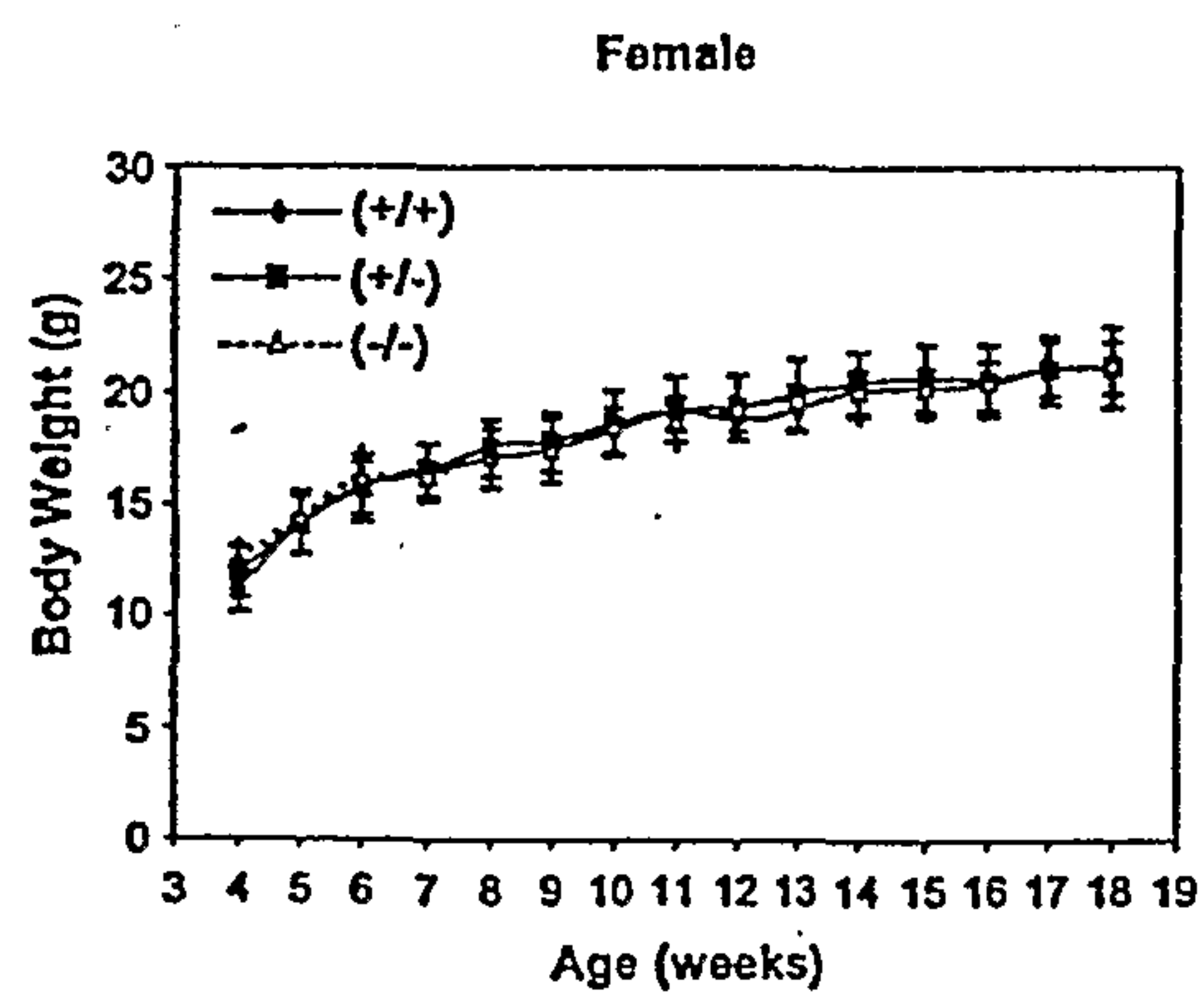


FIG. 2B

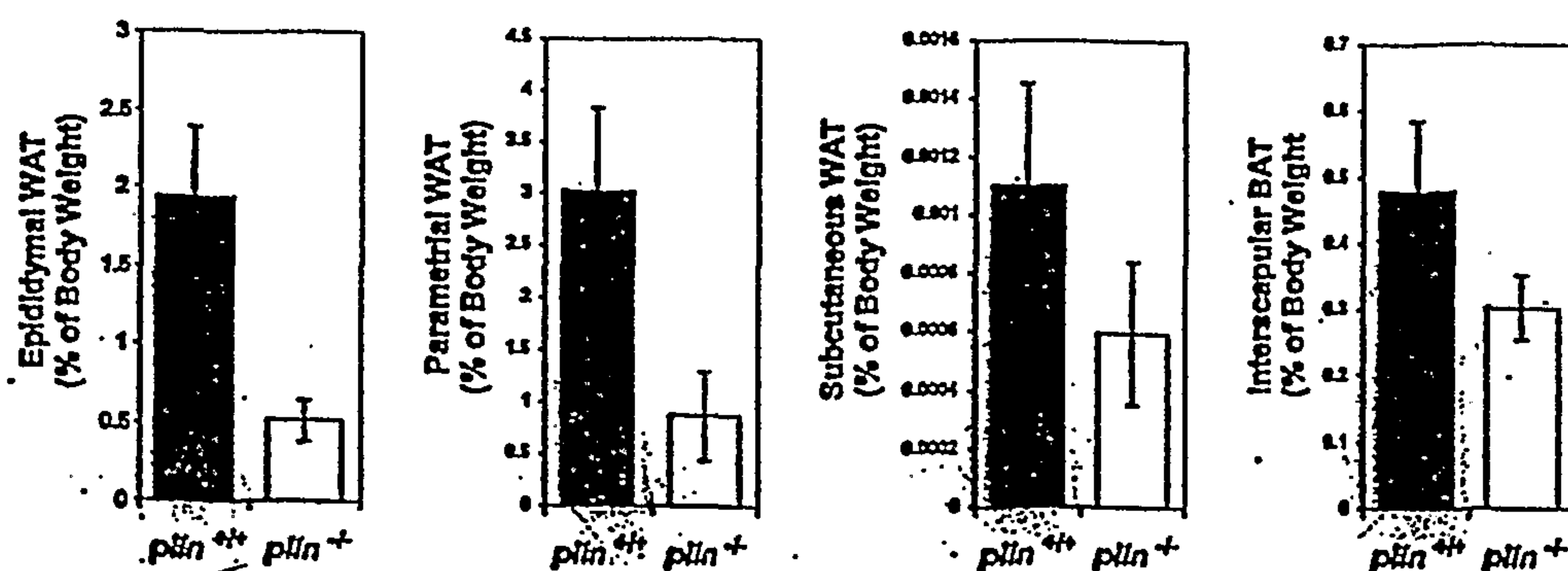


FIG. 2C

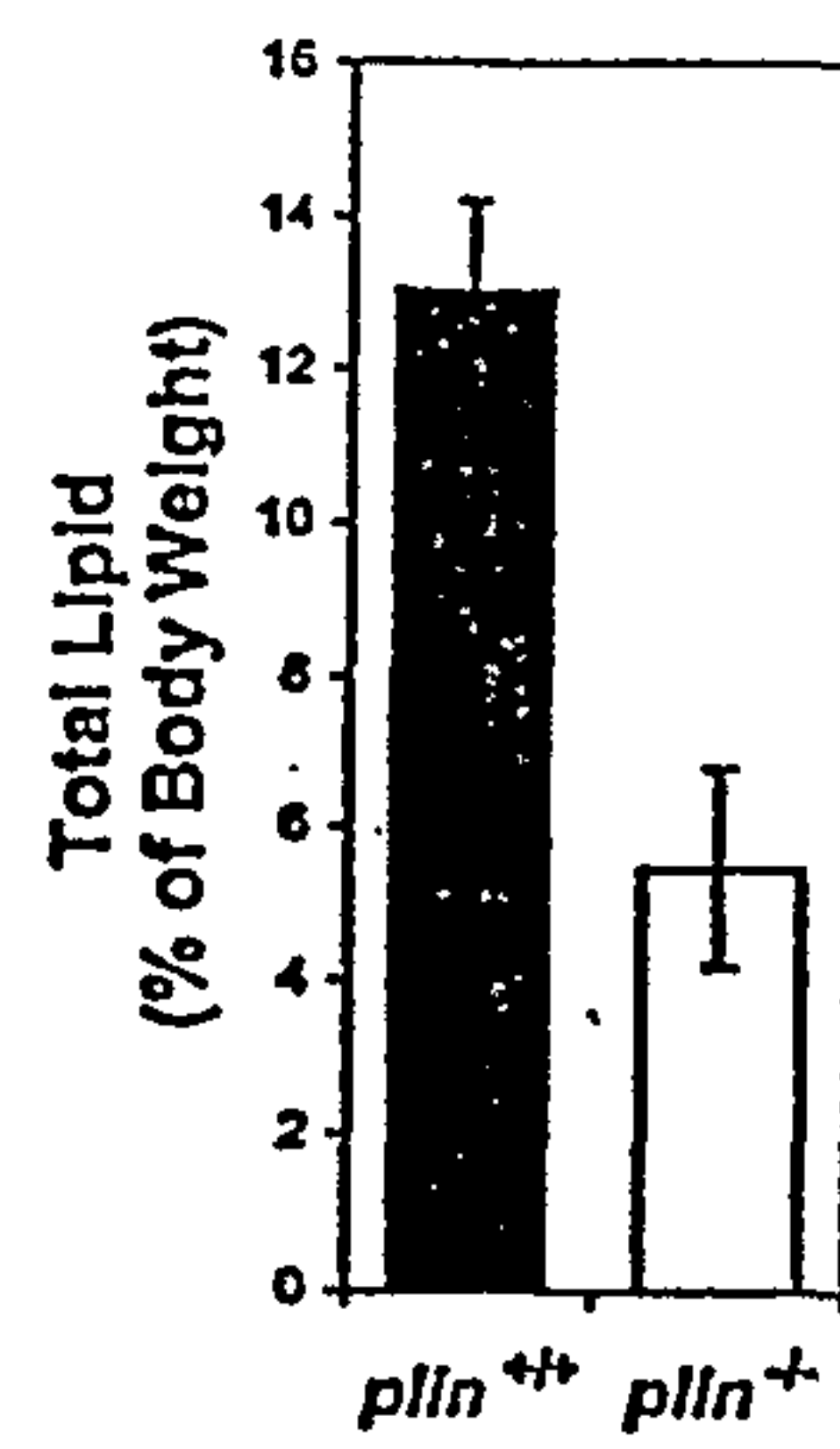


FIG. 2D

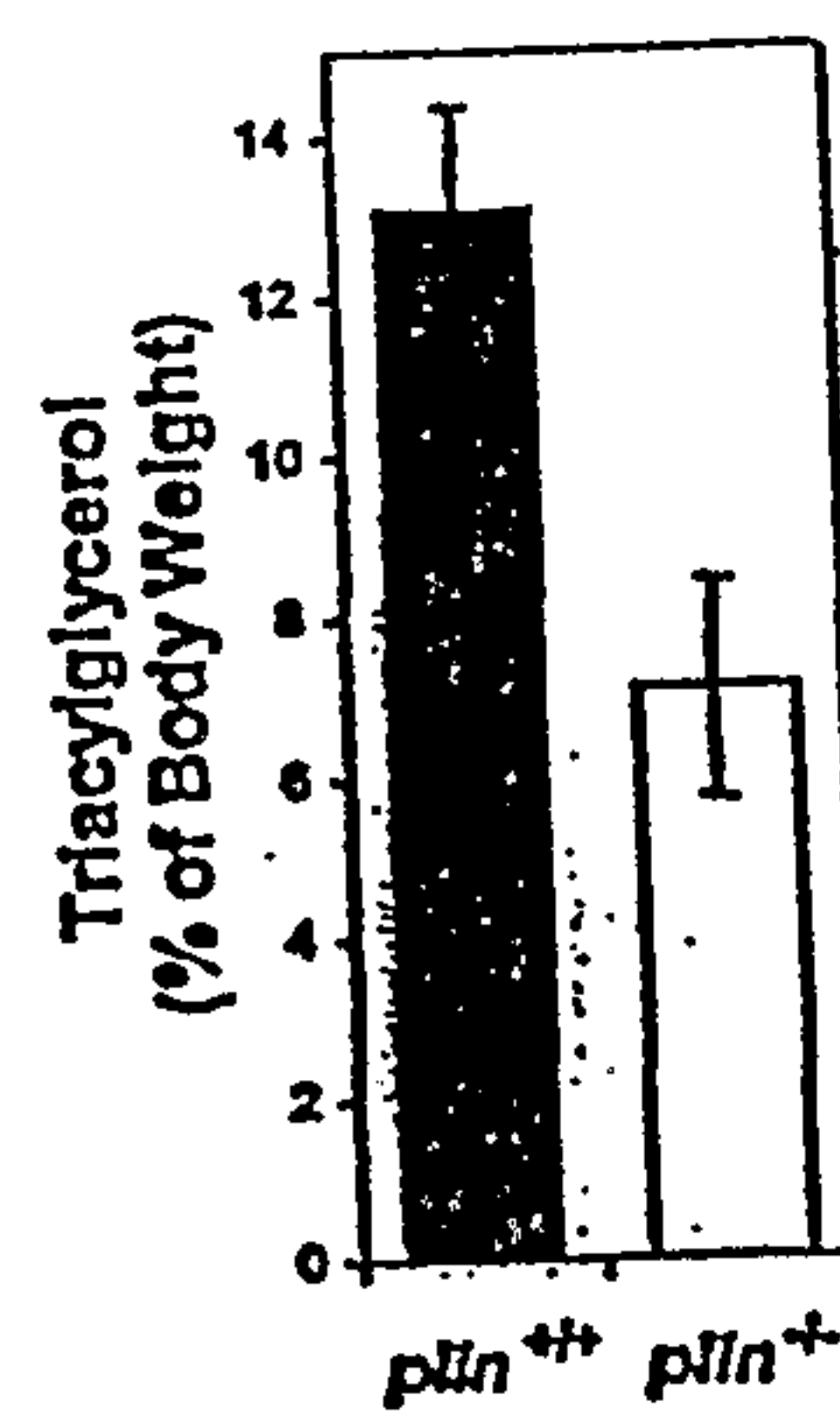


FIG. 2E

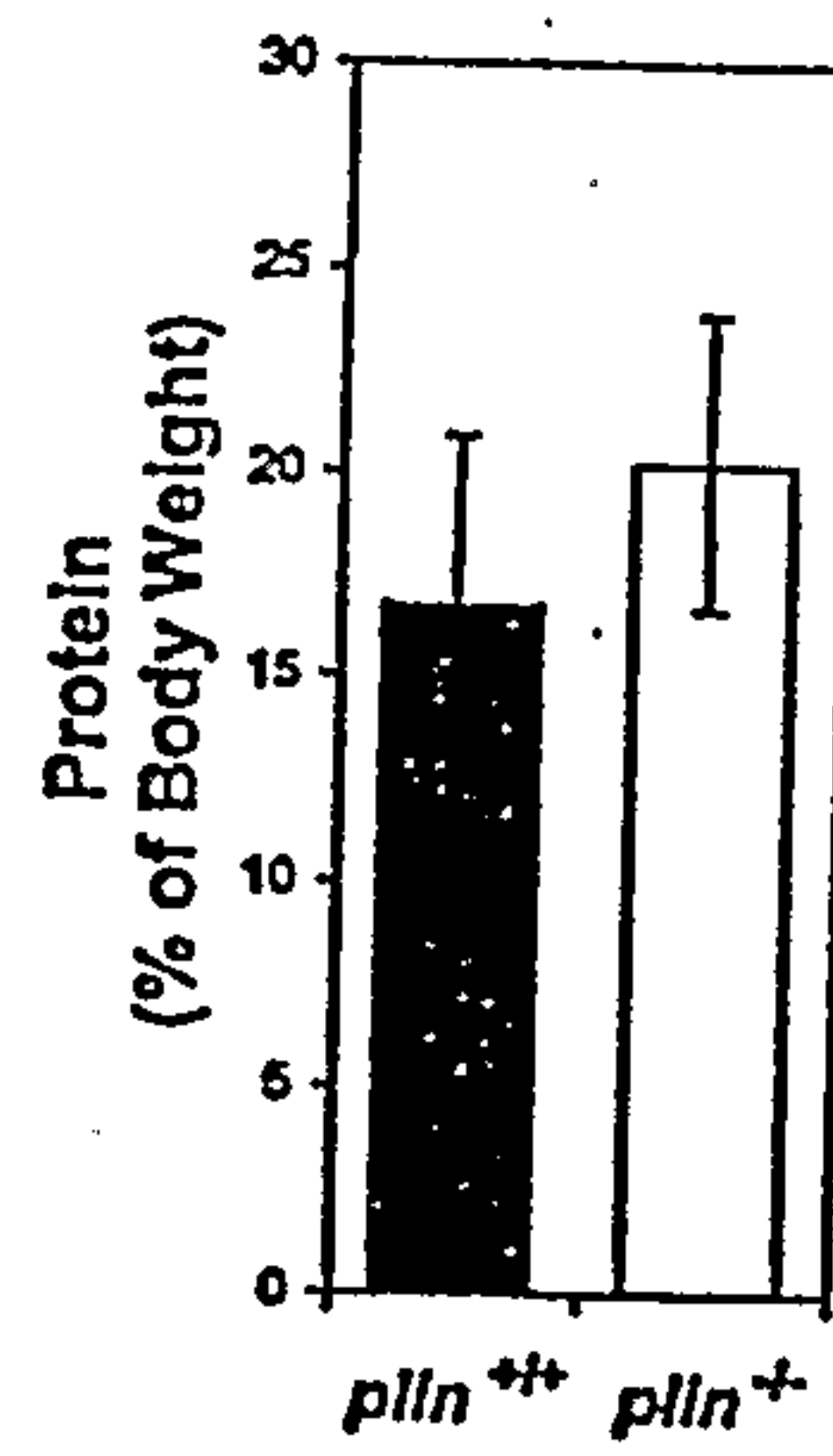


FIG. 2F

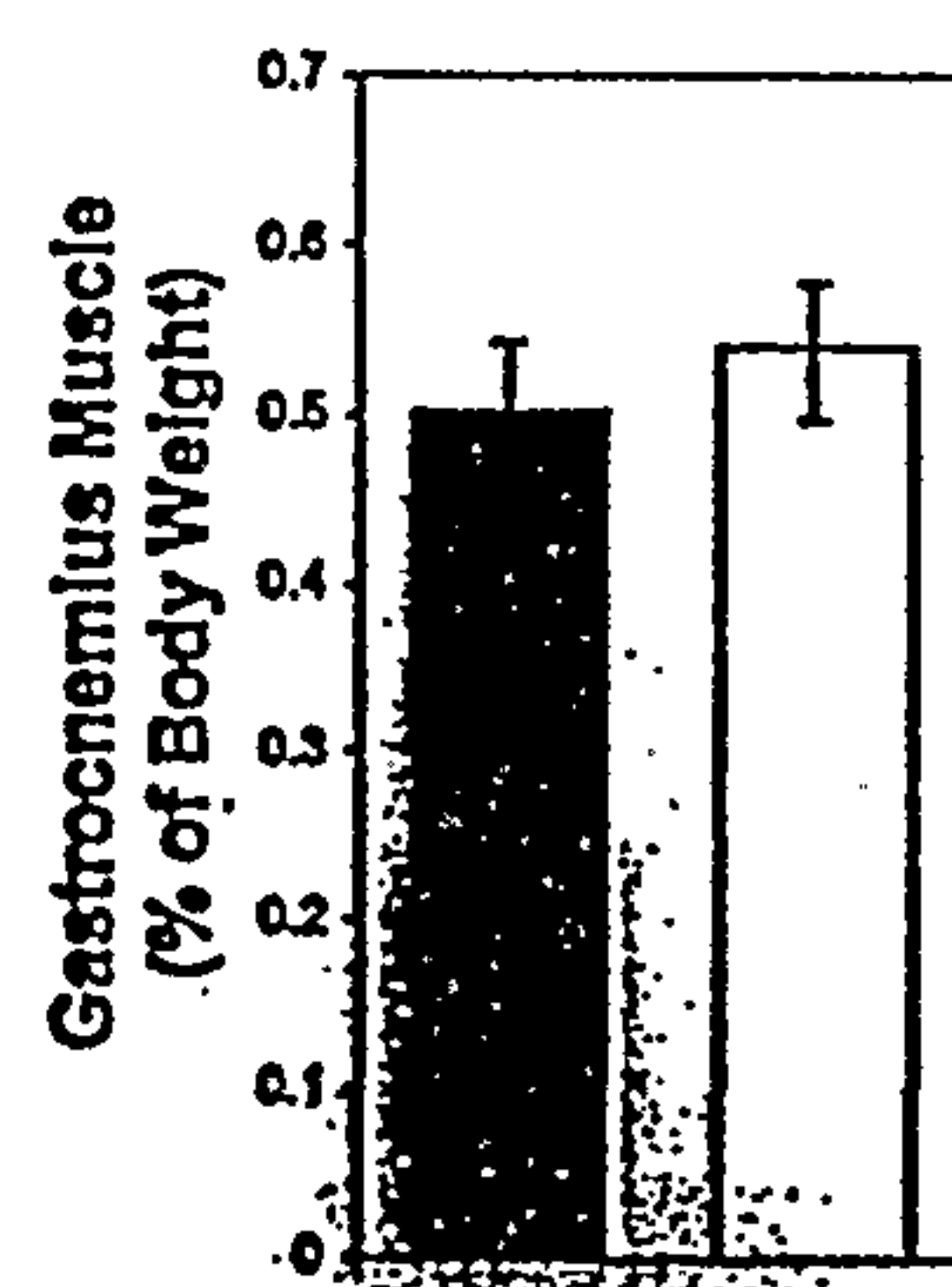
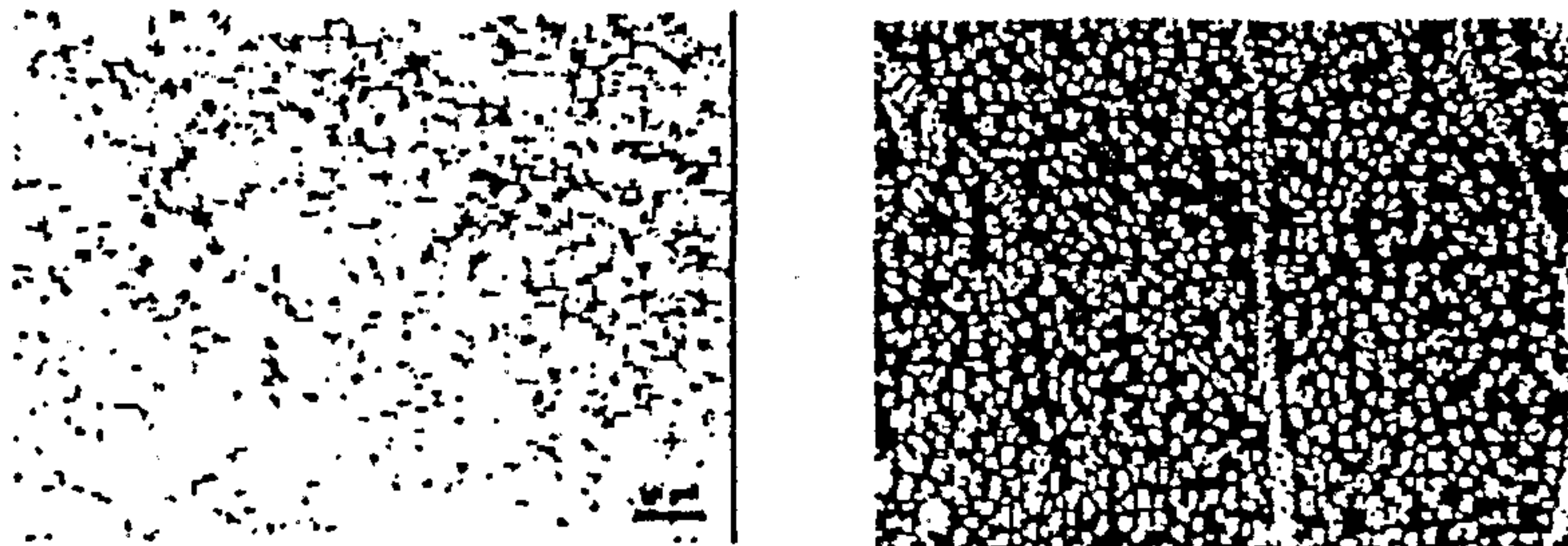
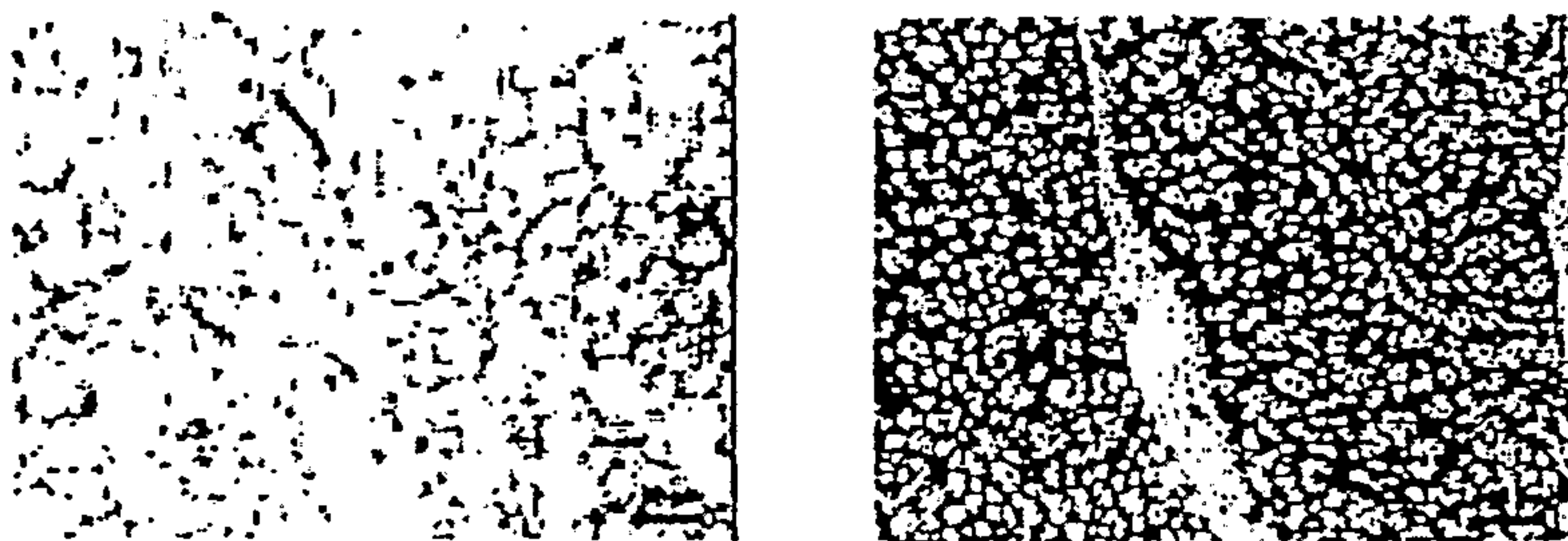
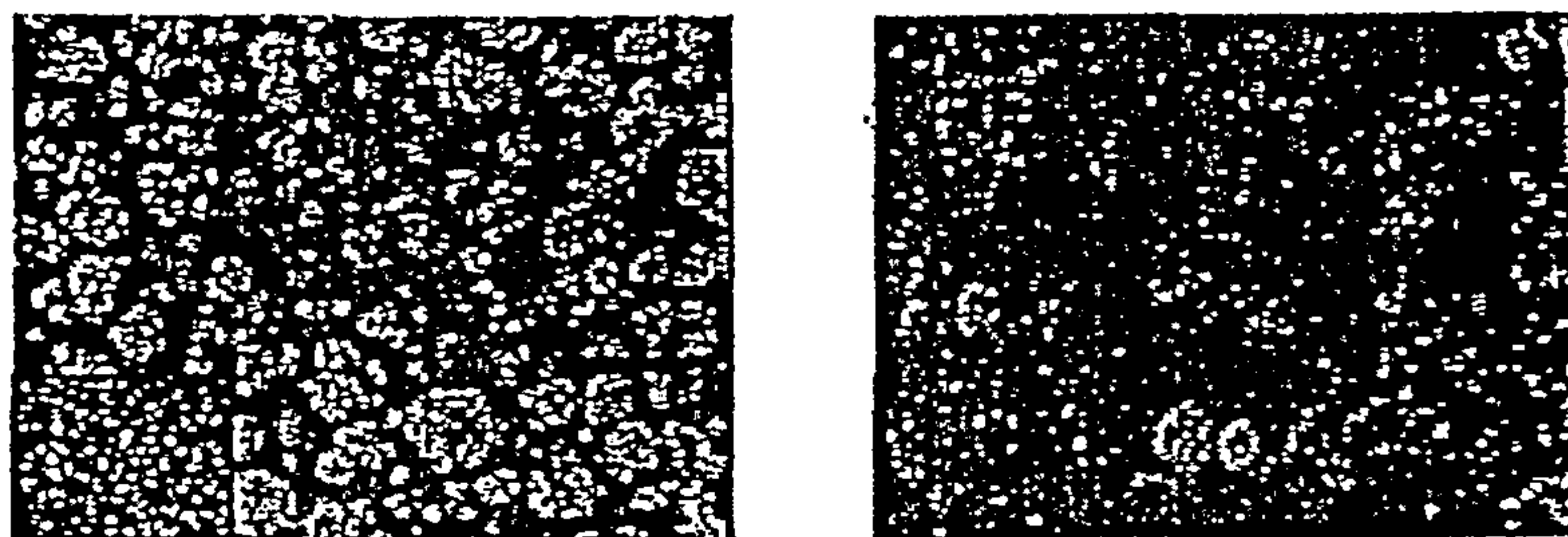
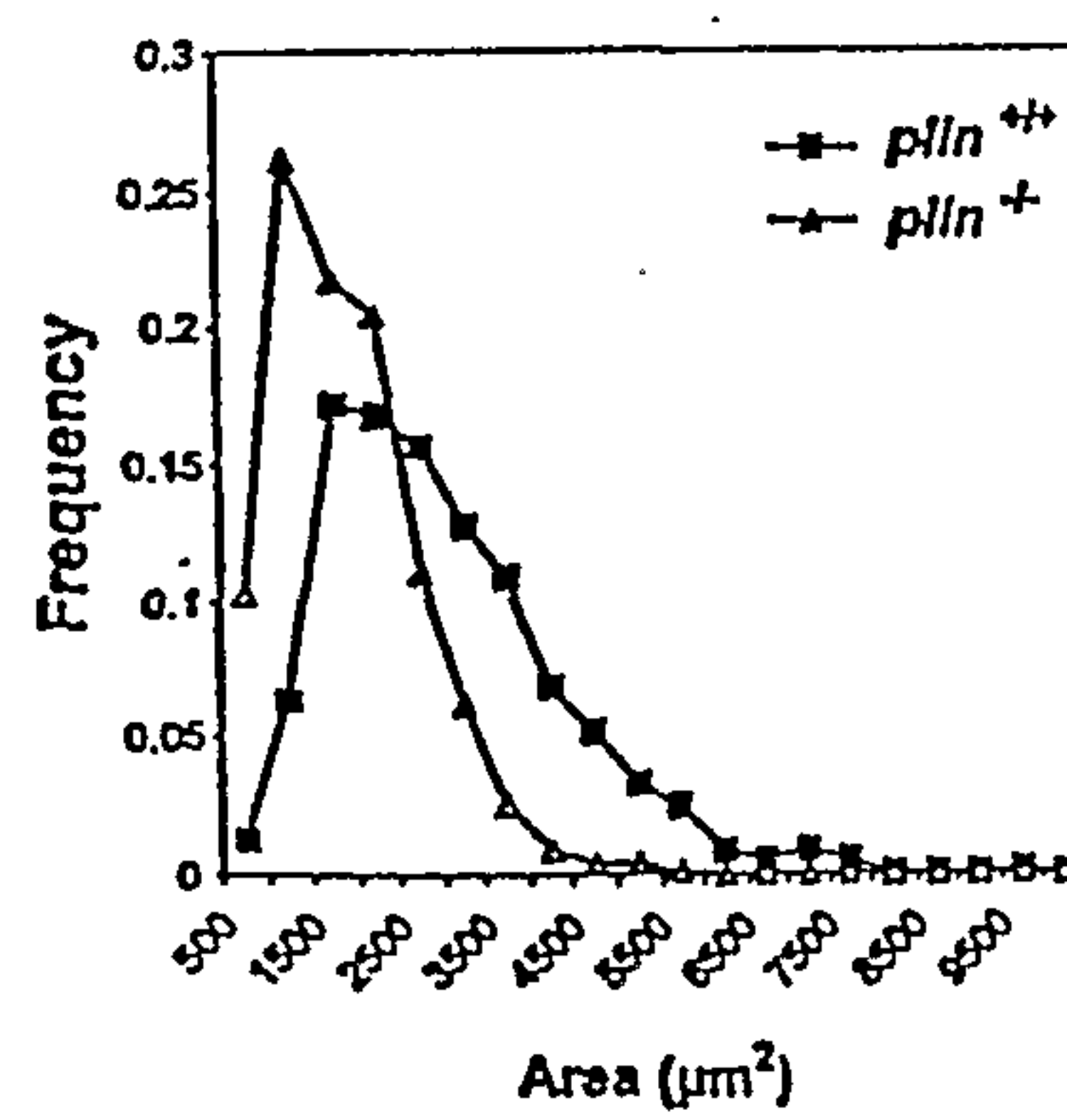


FIG. 2G

Subcutaneous WAT**Epididymal WAT****Interscapular BAT****FIG. 3A****FIG. 3B**

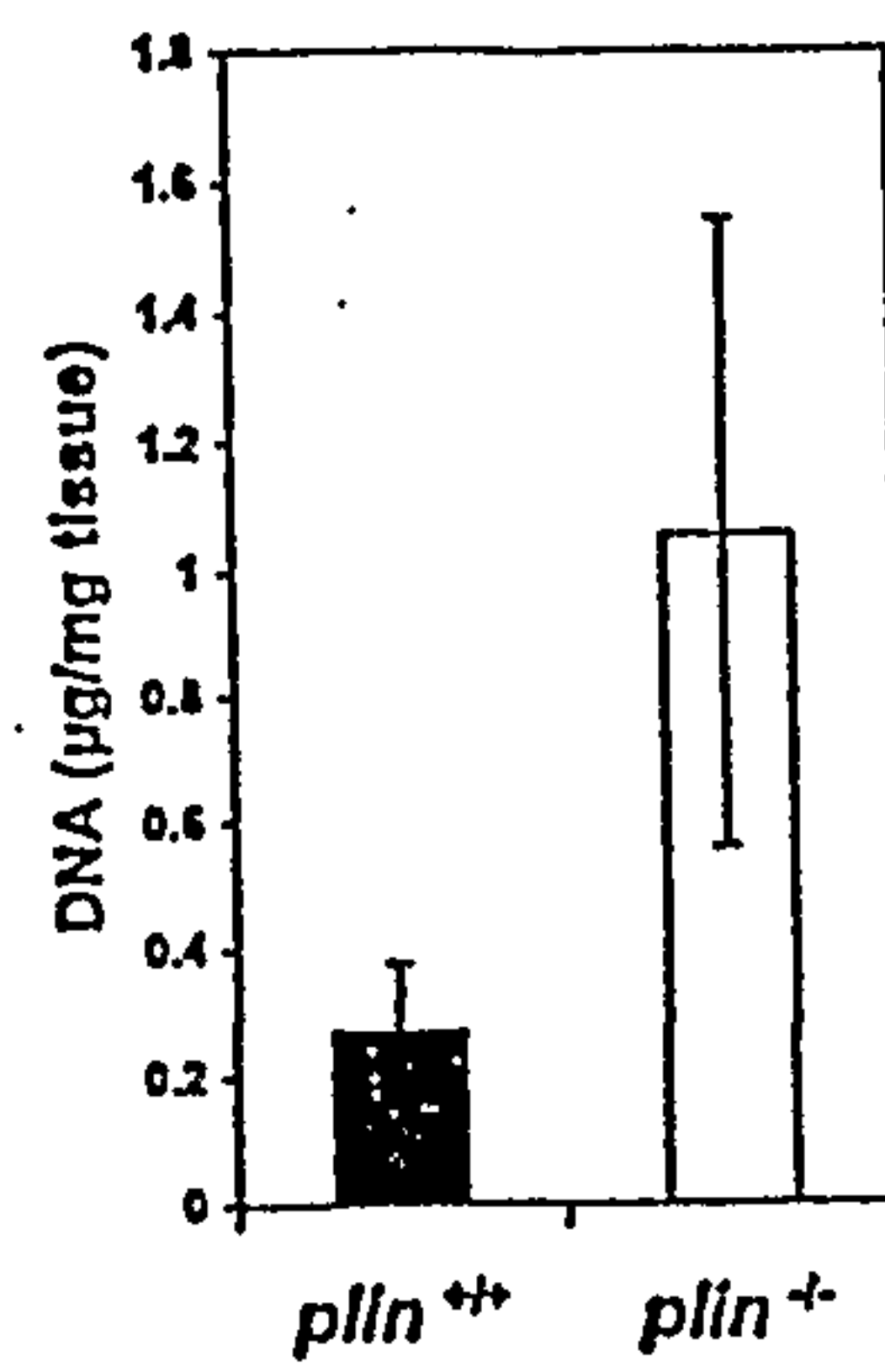


FIG. 3C

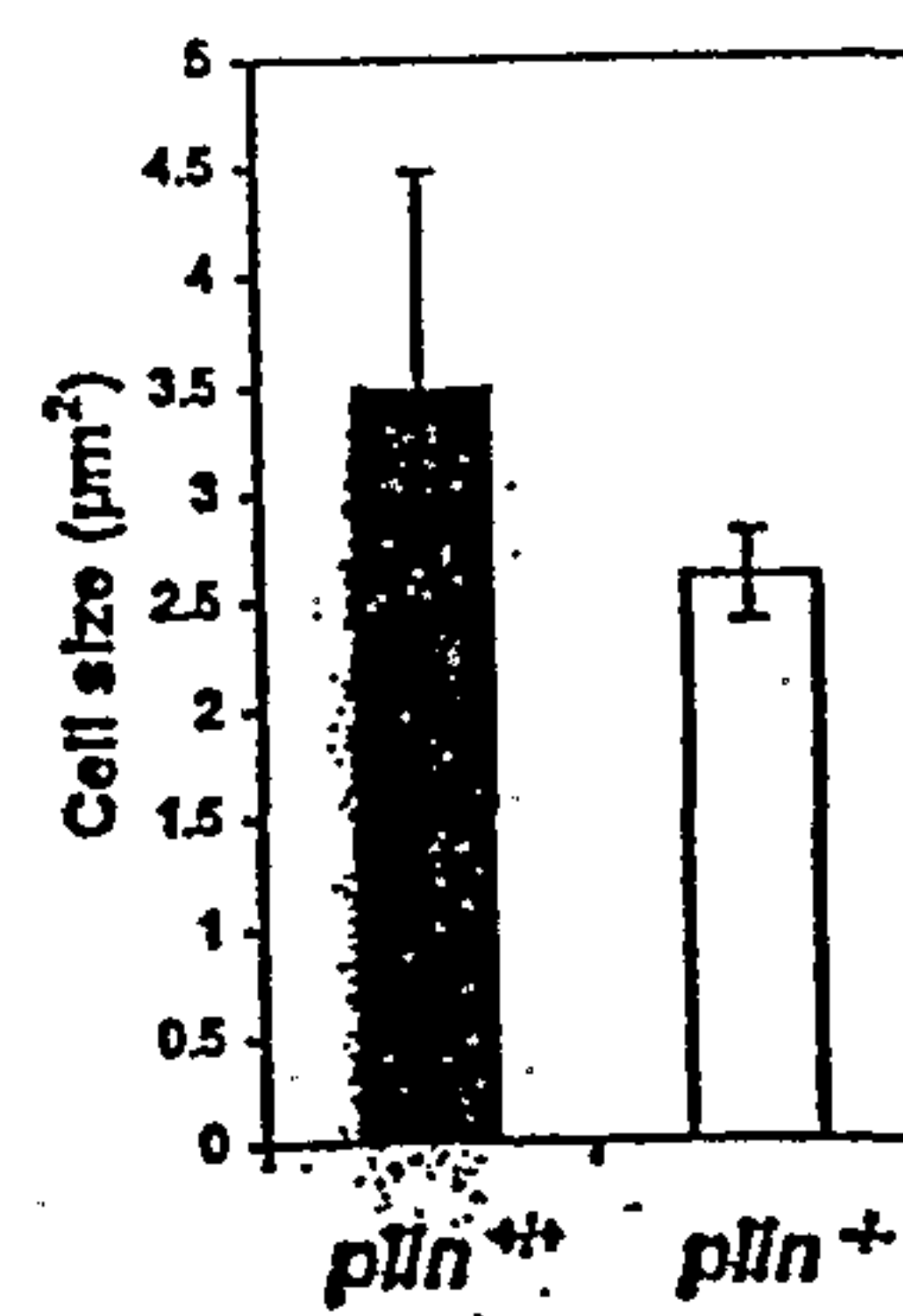


FIG. 3D

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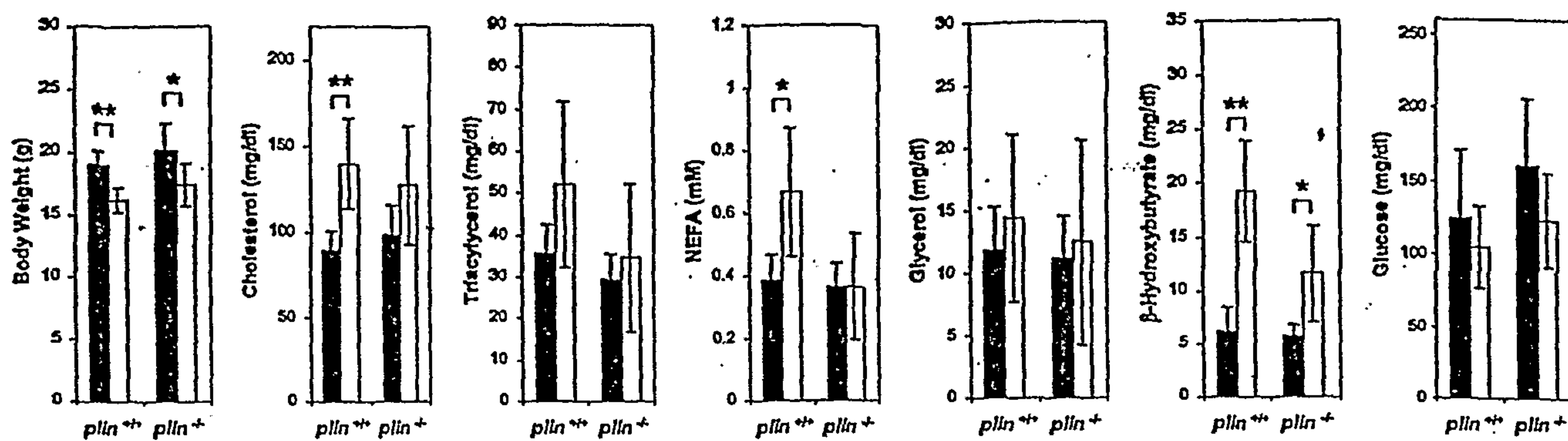


FIG. 4A

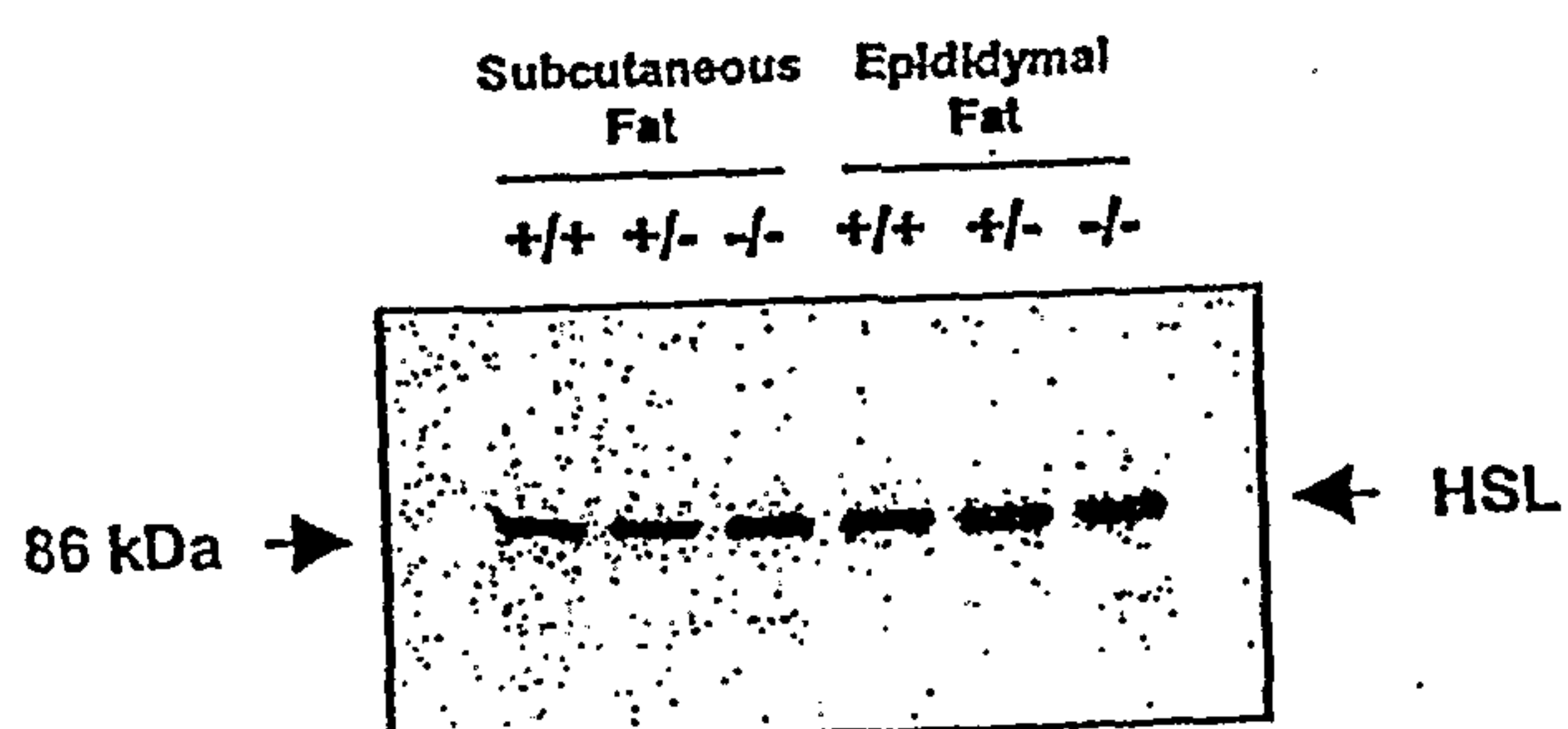


FIG. 4B

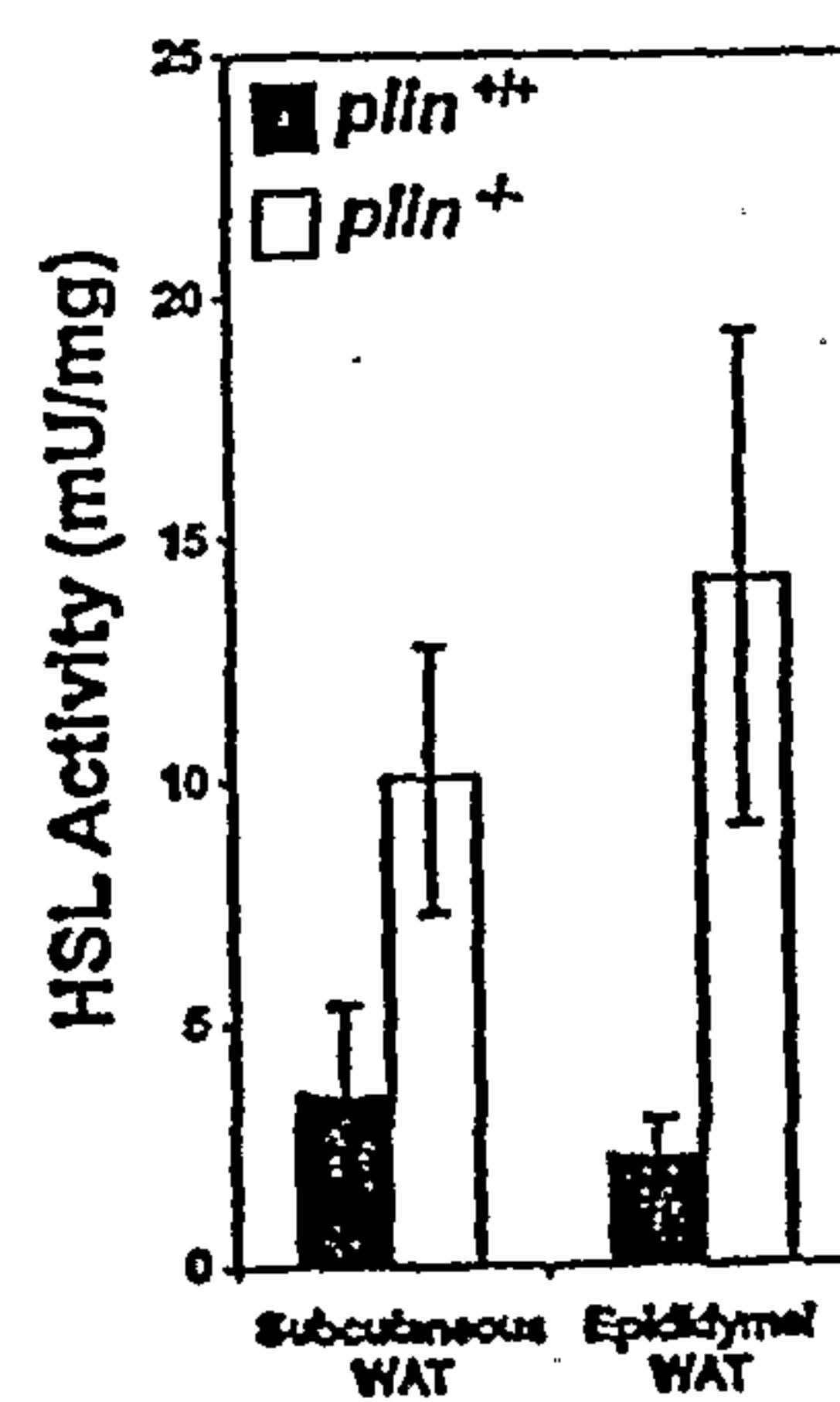


FIG. 4C

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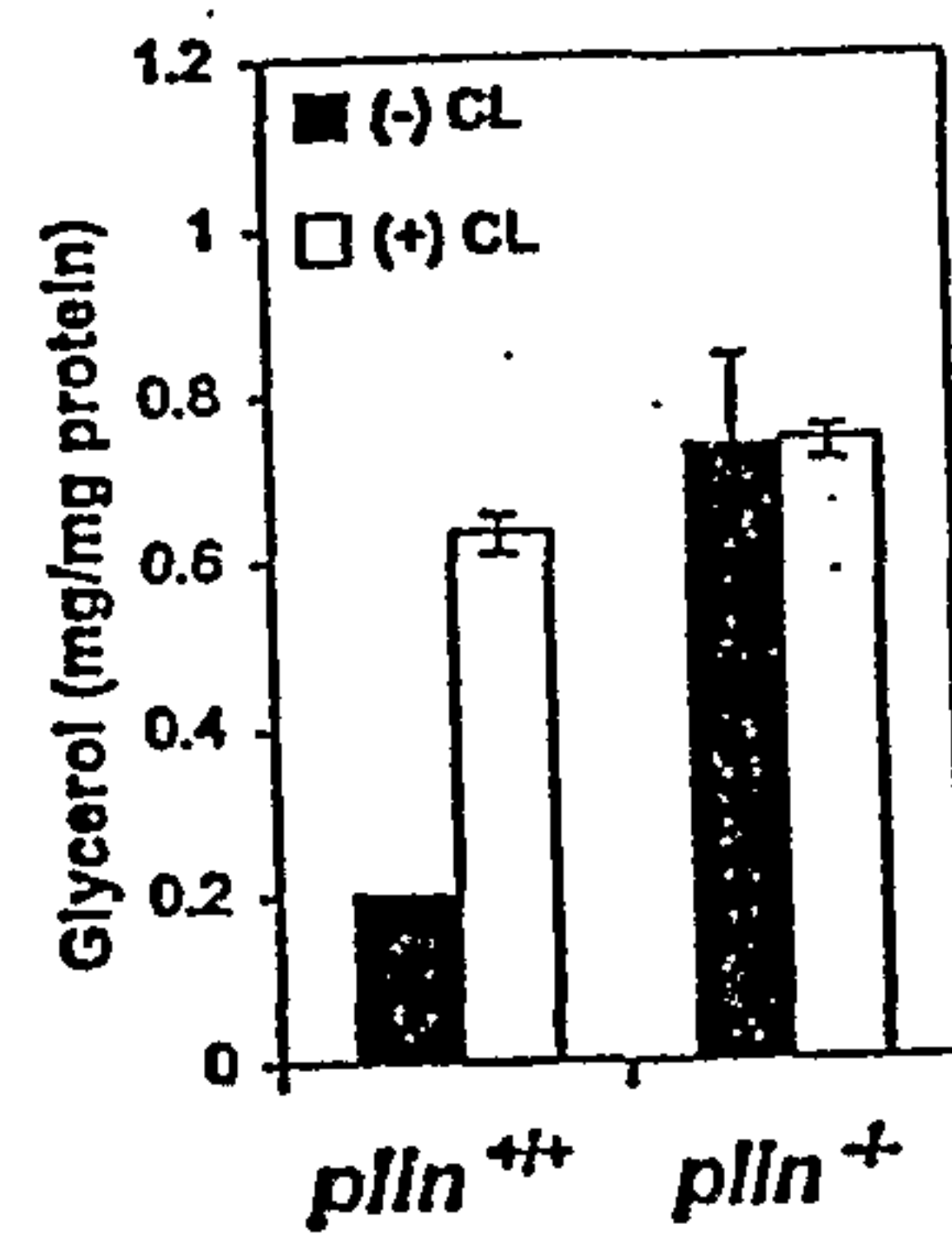


FIG. 4D

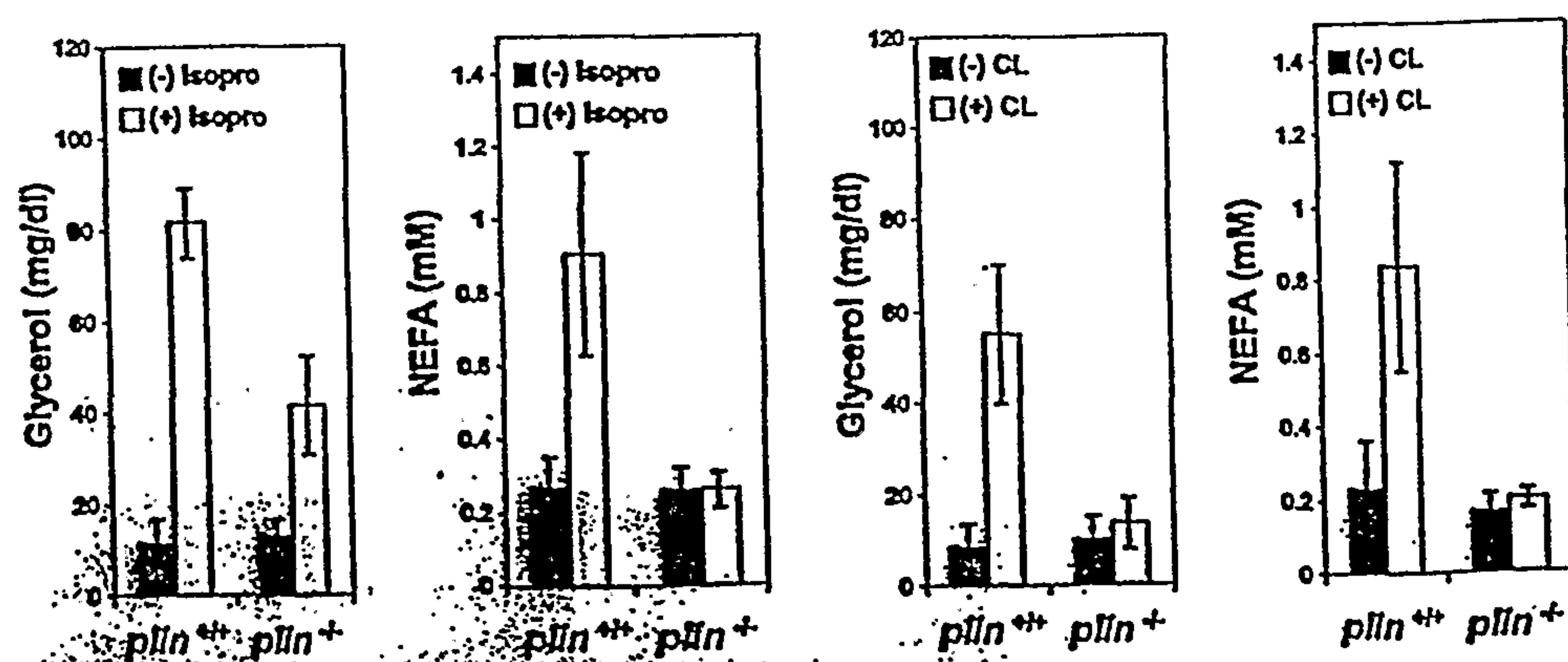


FIG. 4E

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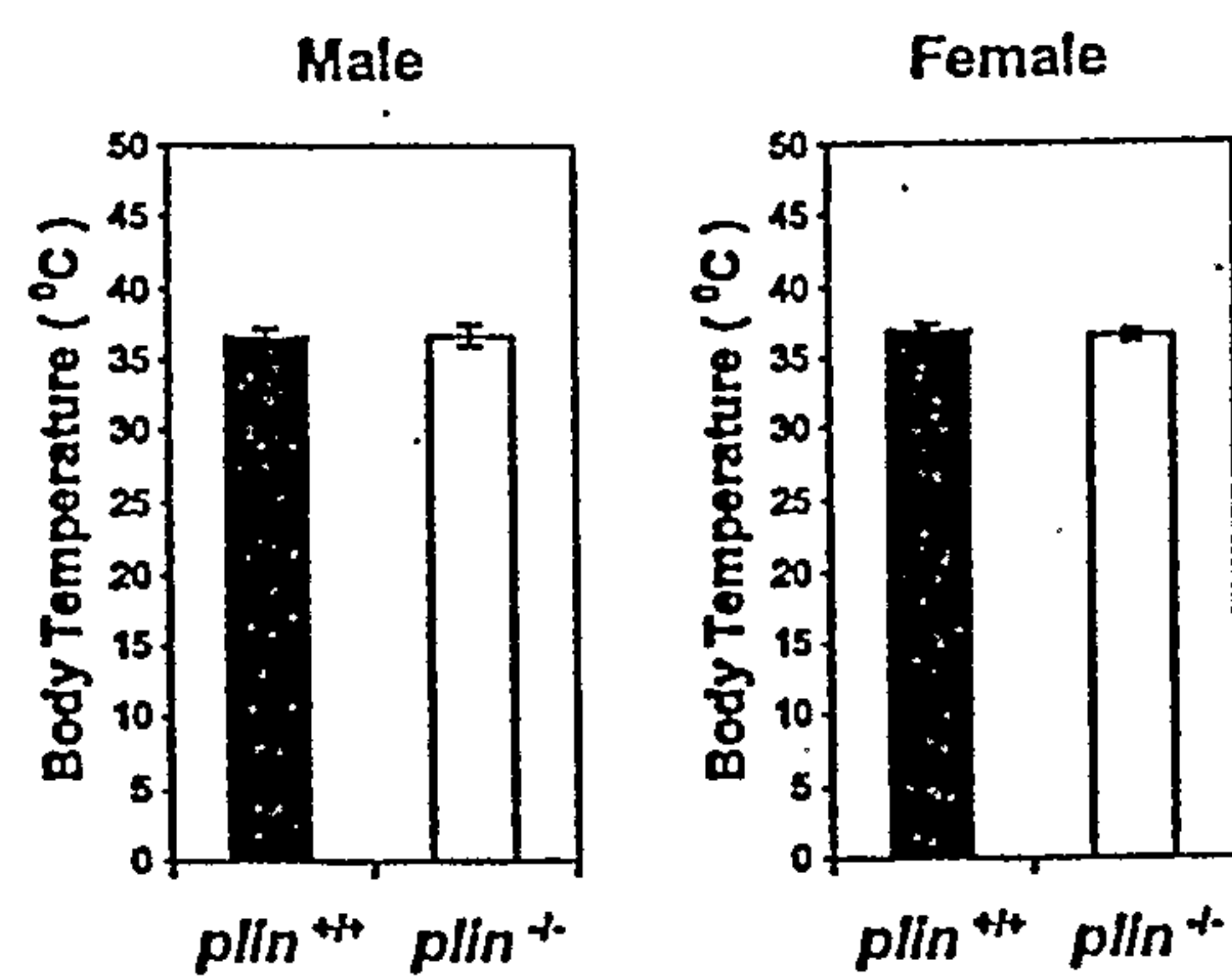


FIG. 5A

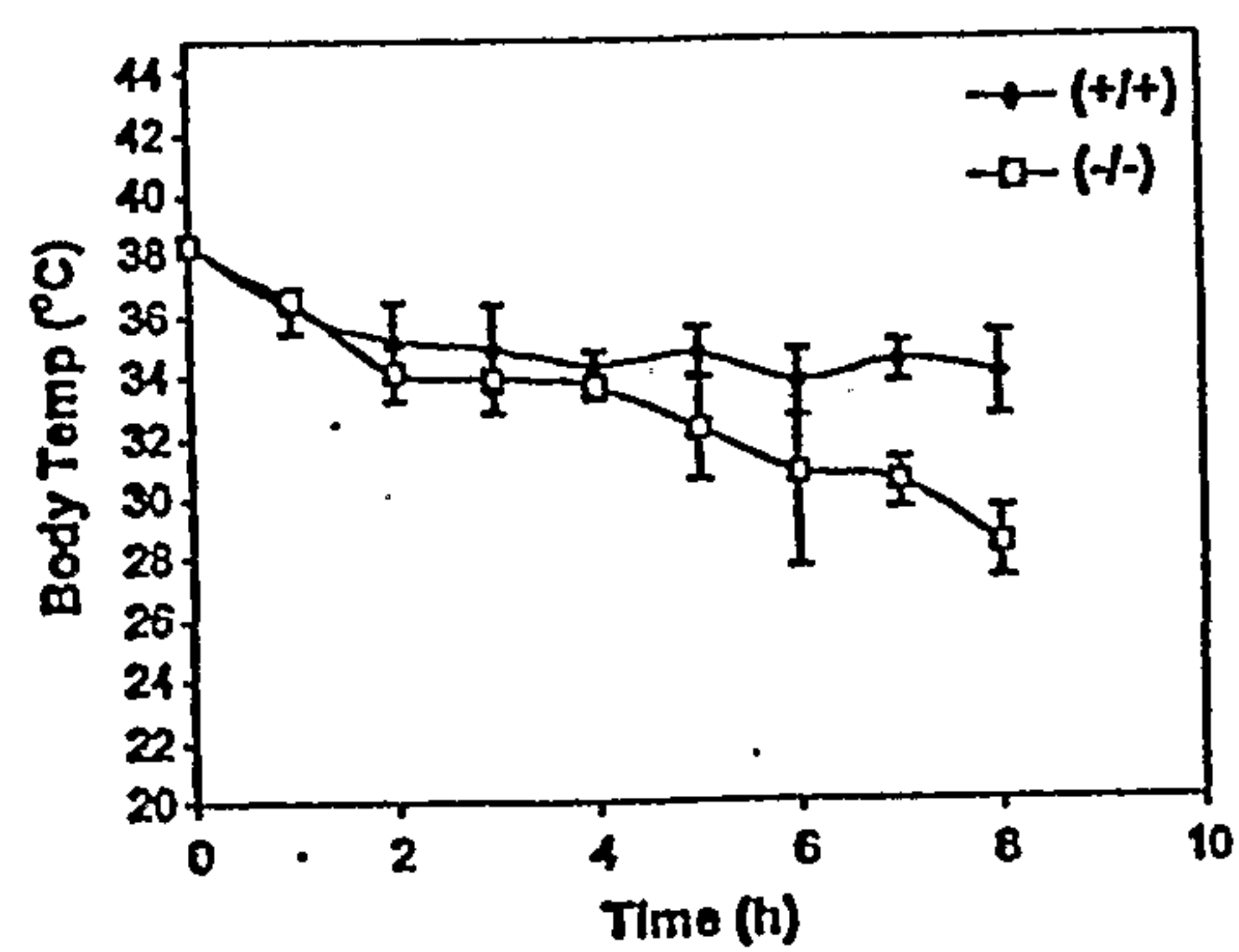


FIG. 5B

10/26

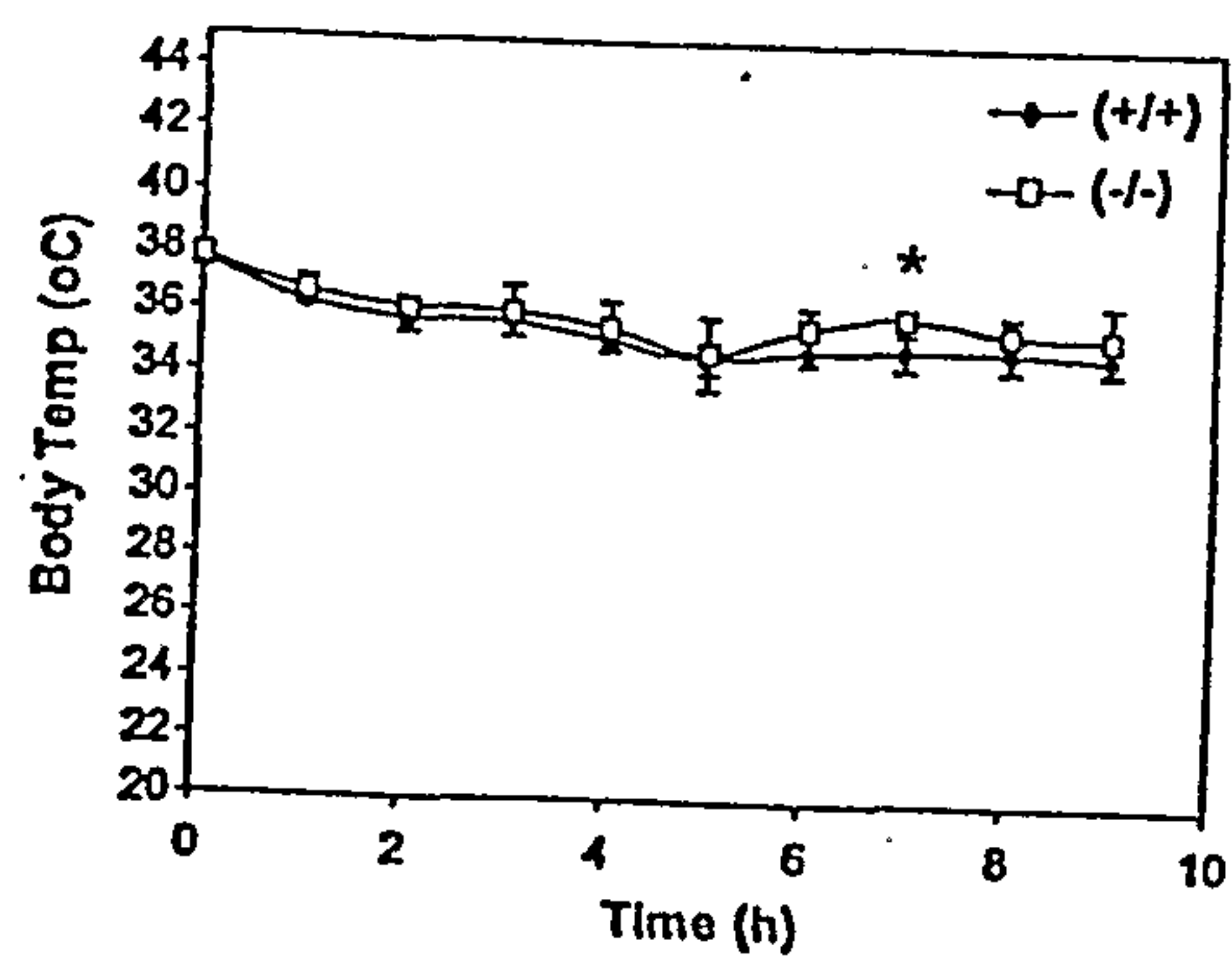


FIG. 5C

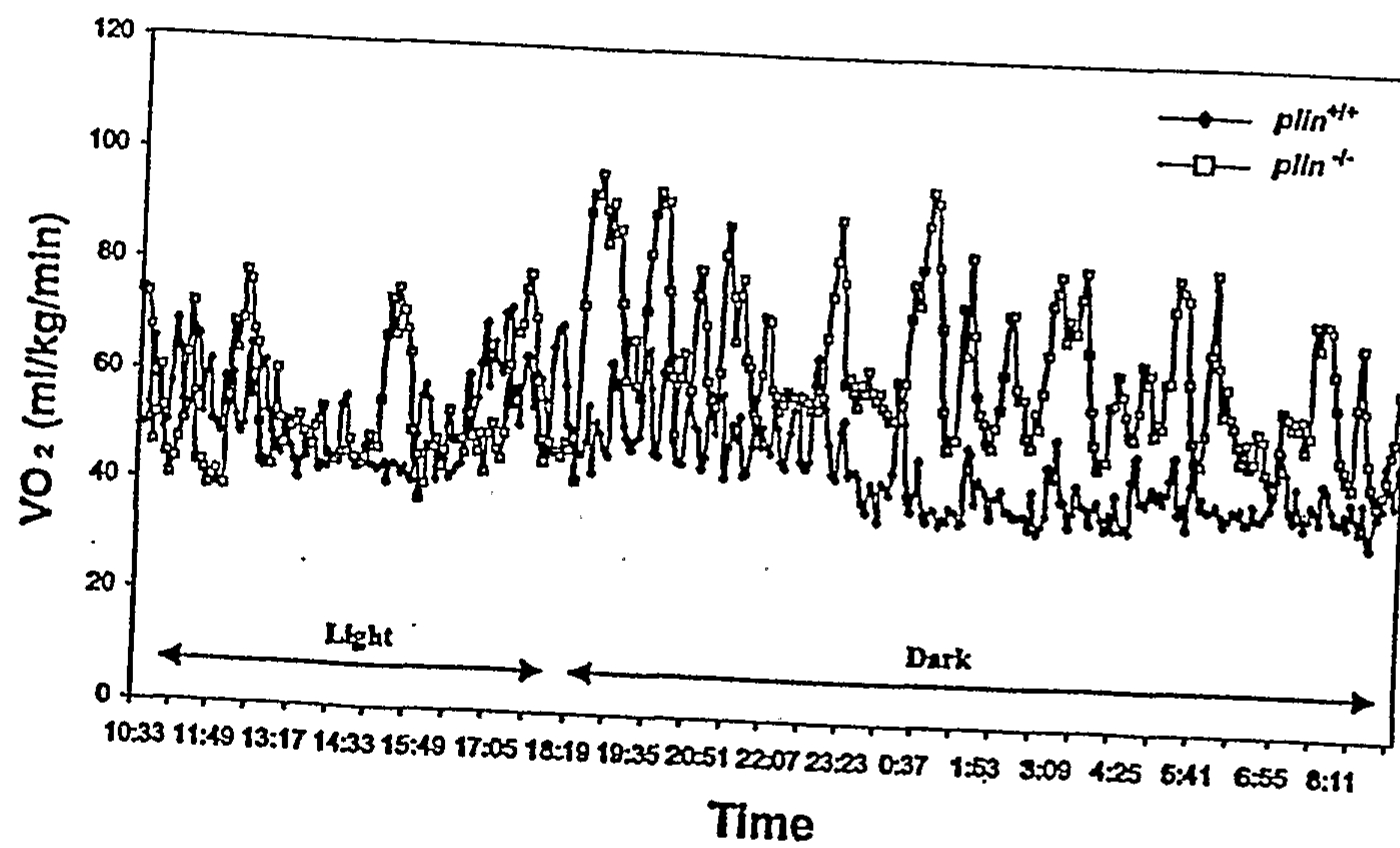


FIG. 5D

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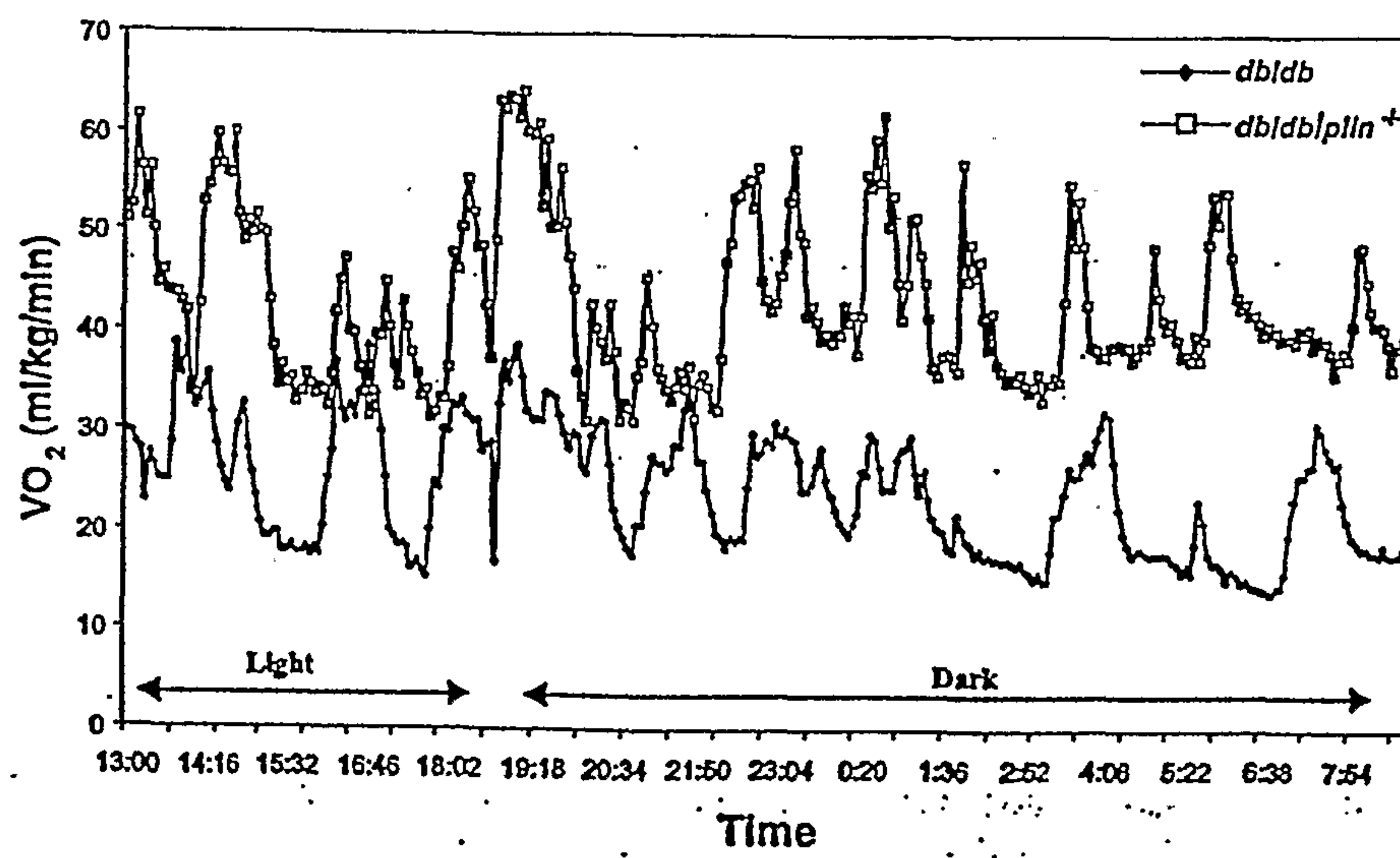


FIG. 5E

12/26

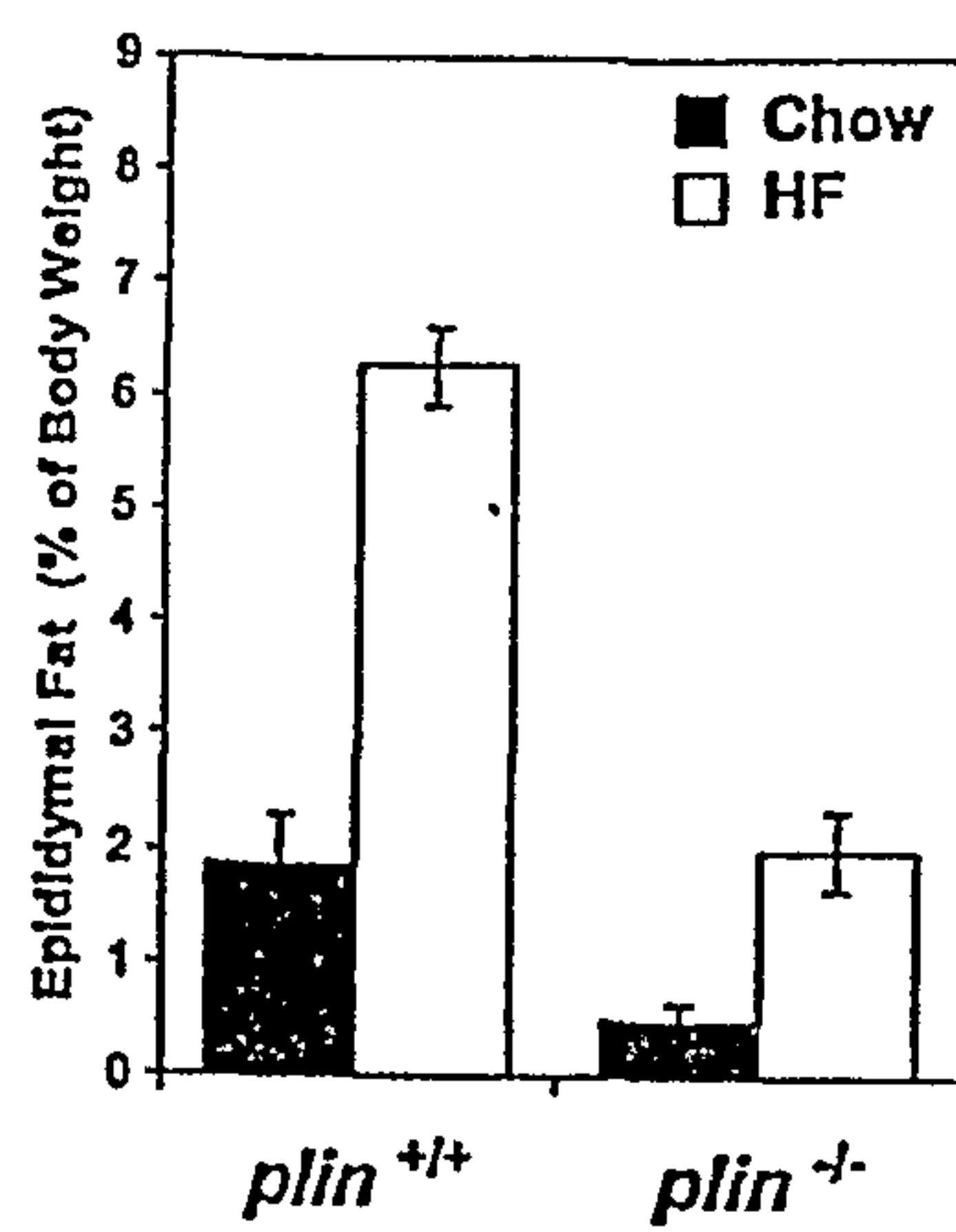


FIG. 6A

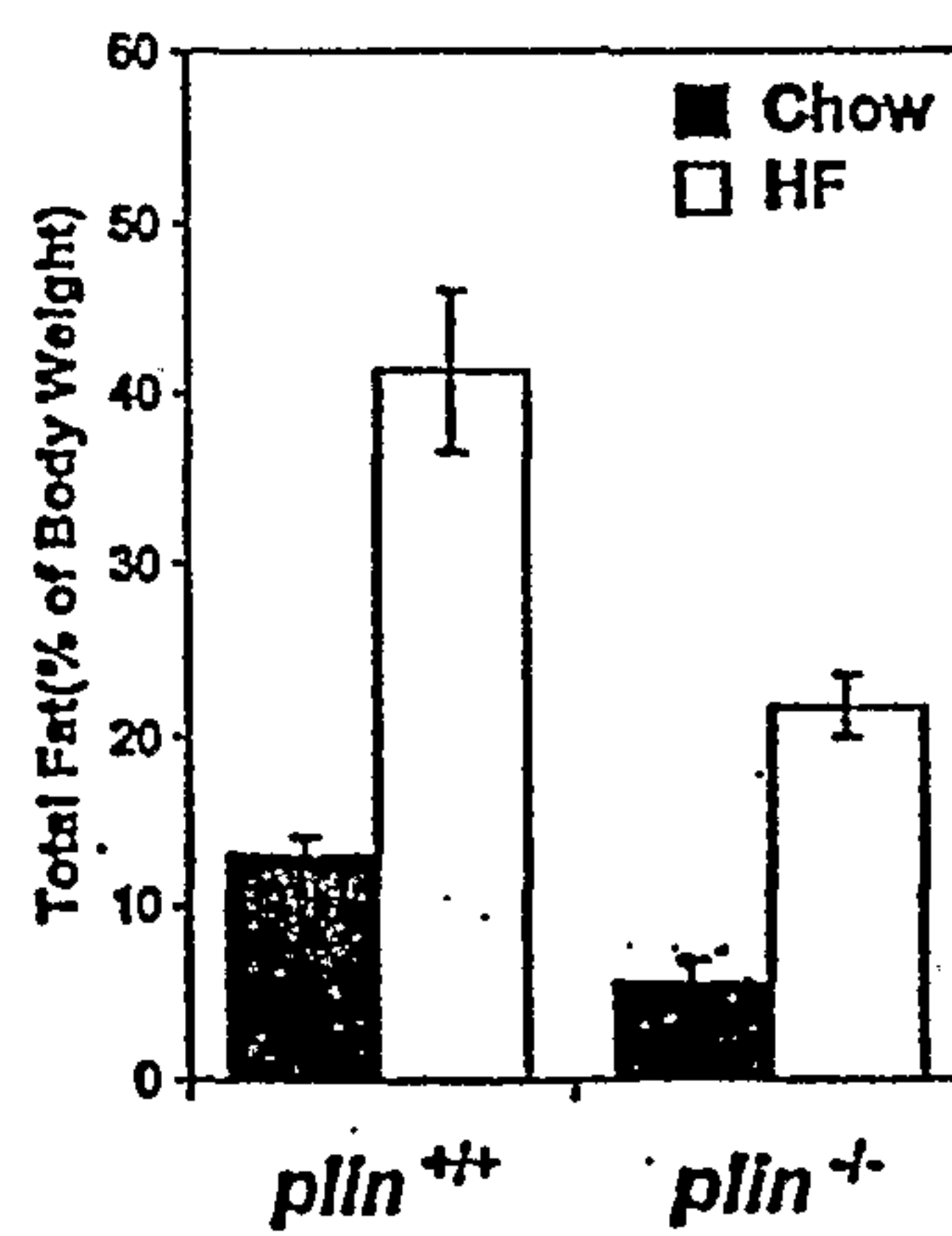


FIG. 6B

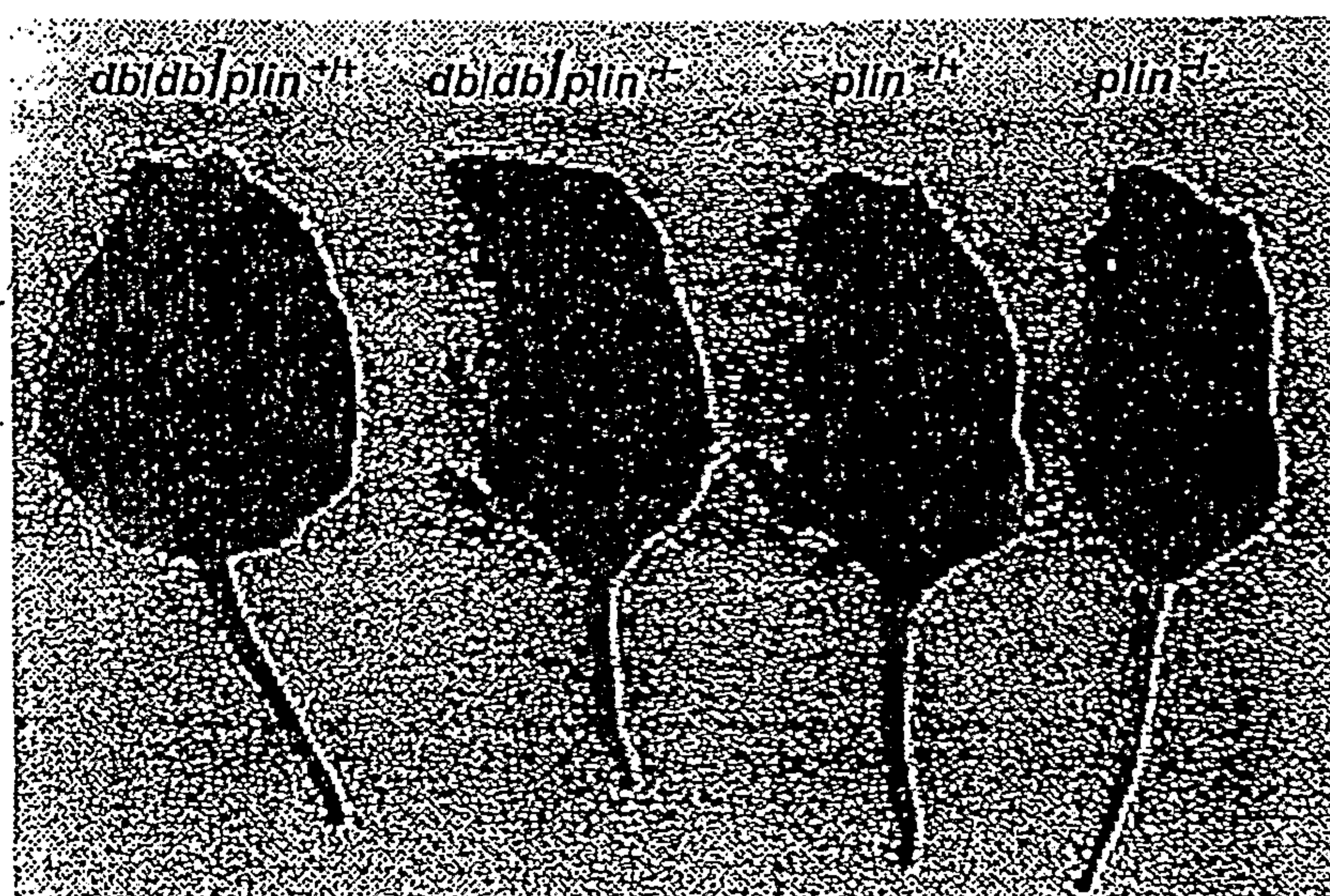


FIG. 6C

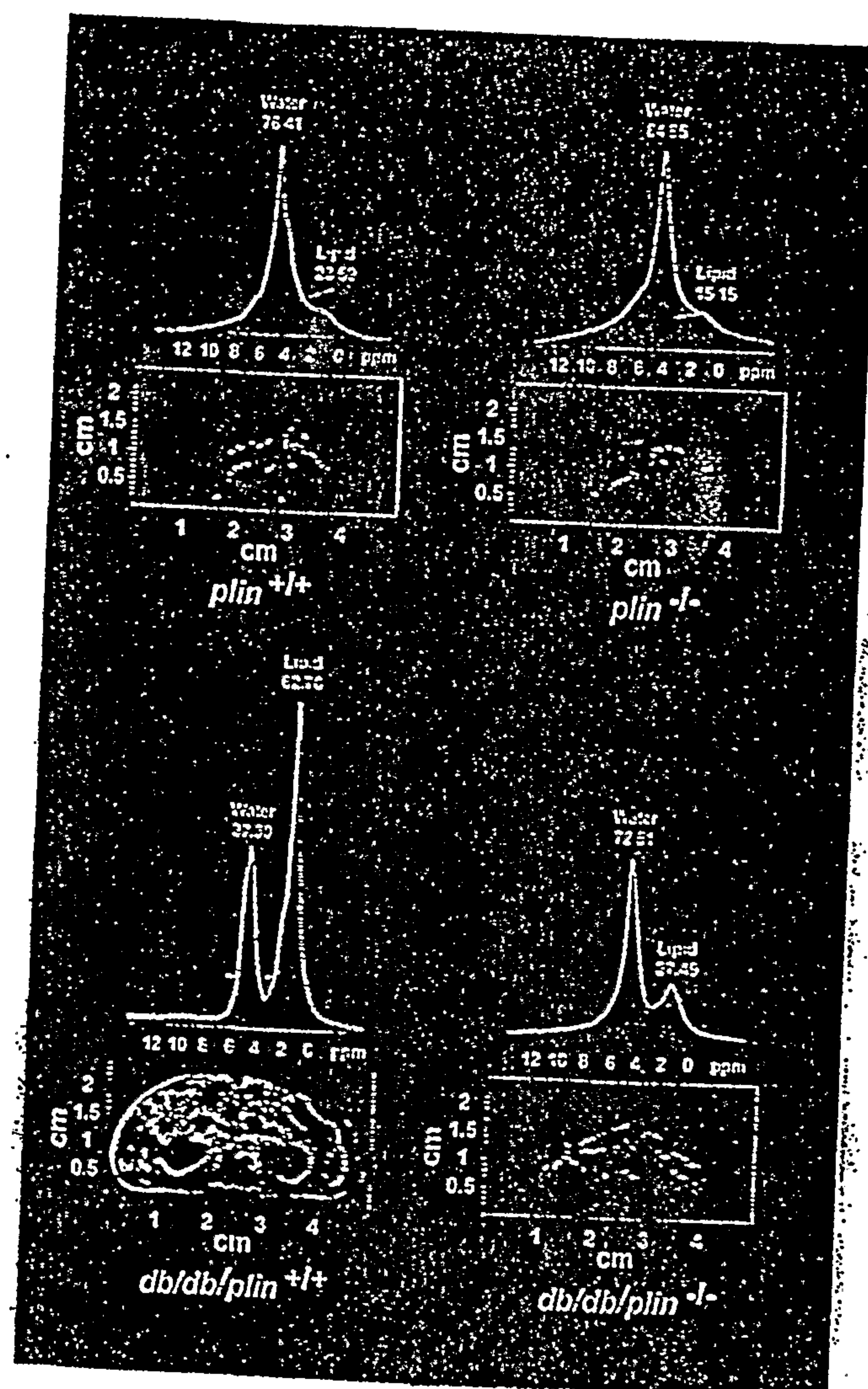


FIG. 6D

Body Weight of Male *Lepr^{db/db} / plin^{-/-}*

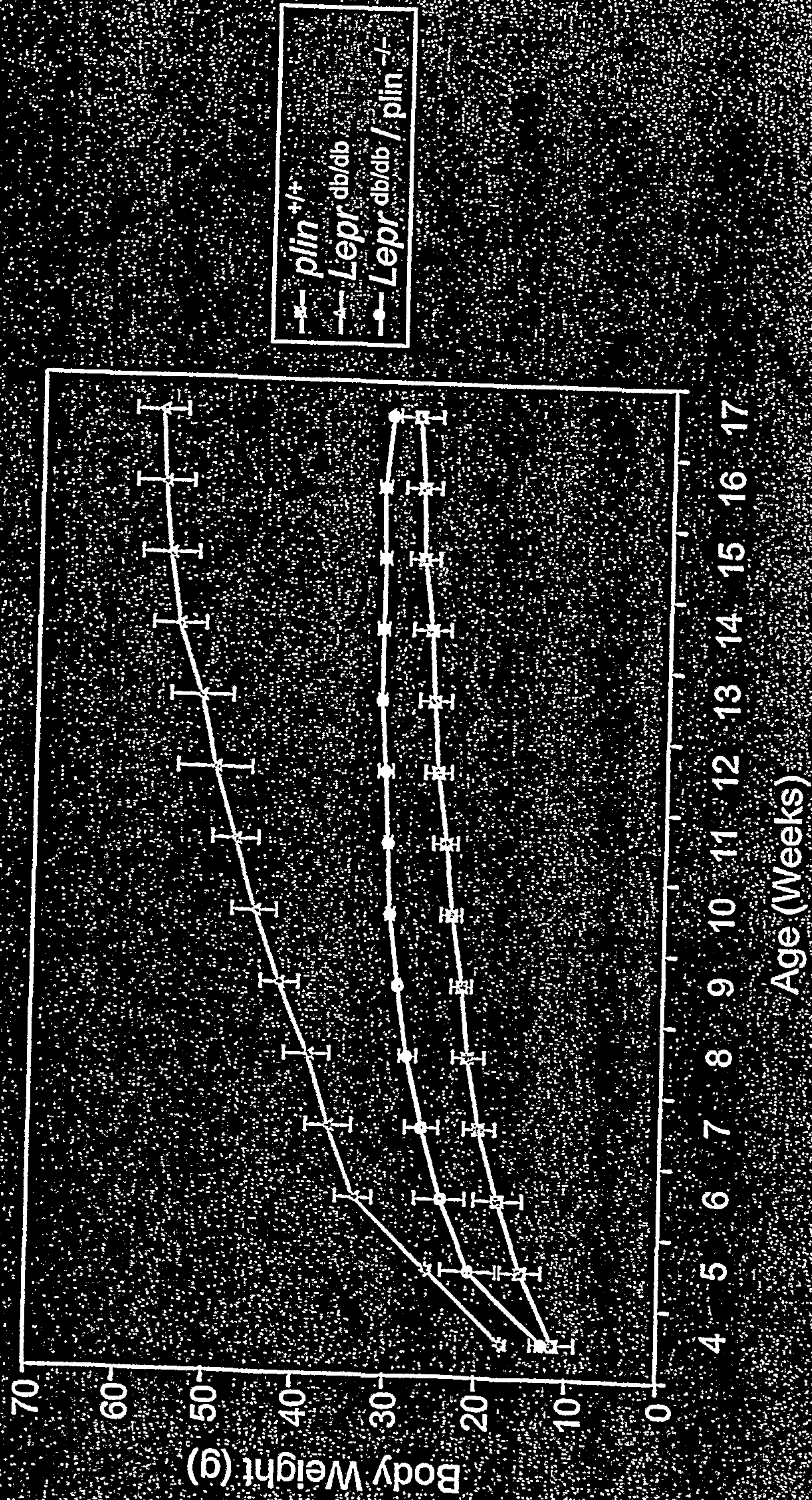


FIG. 7A

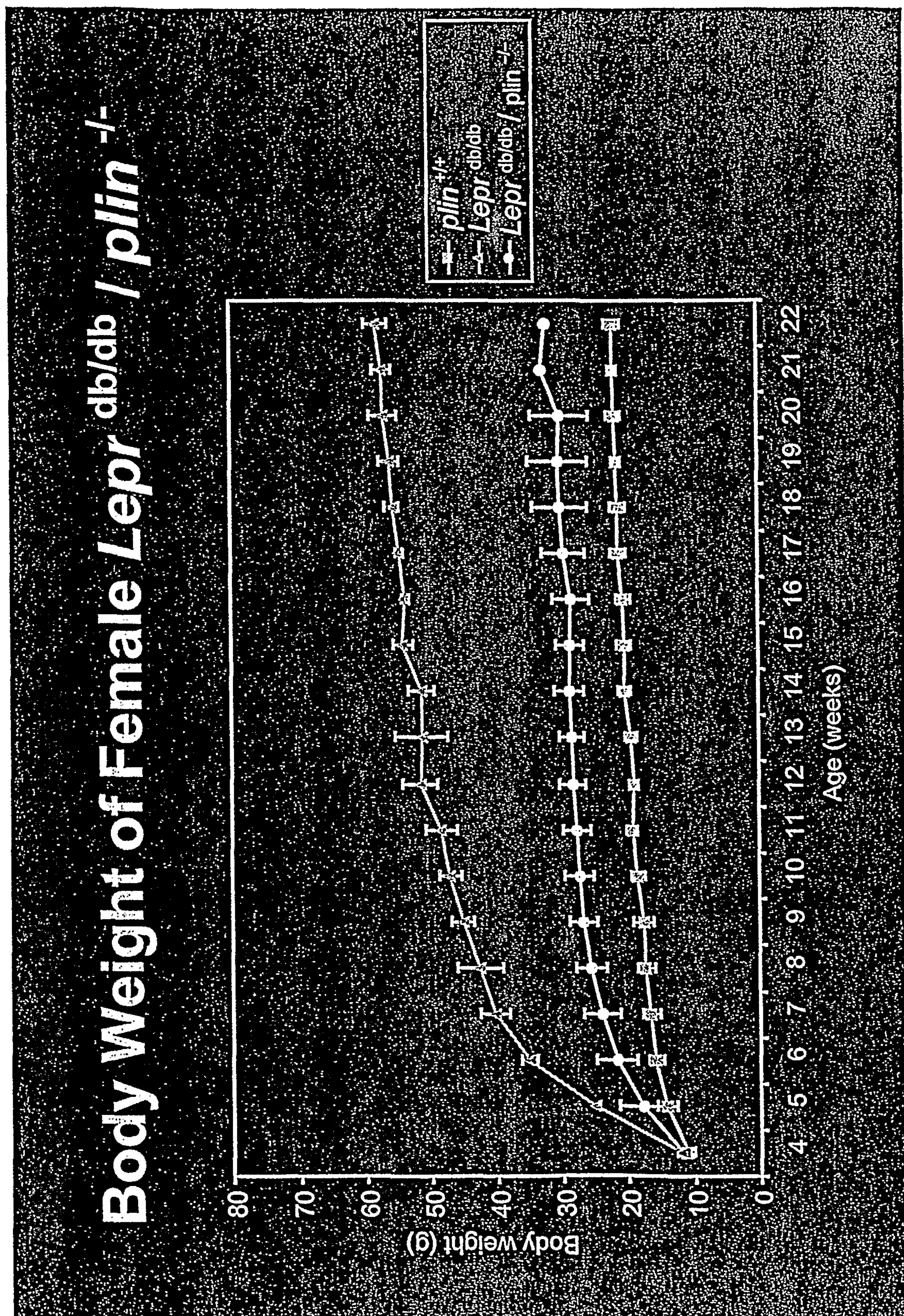


FIG. 7B

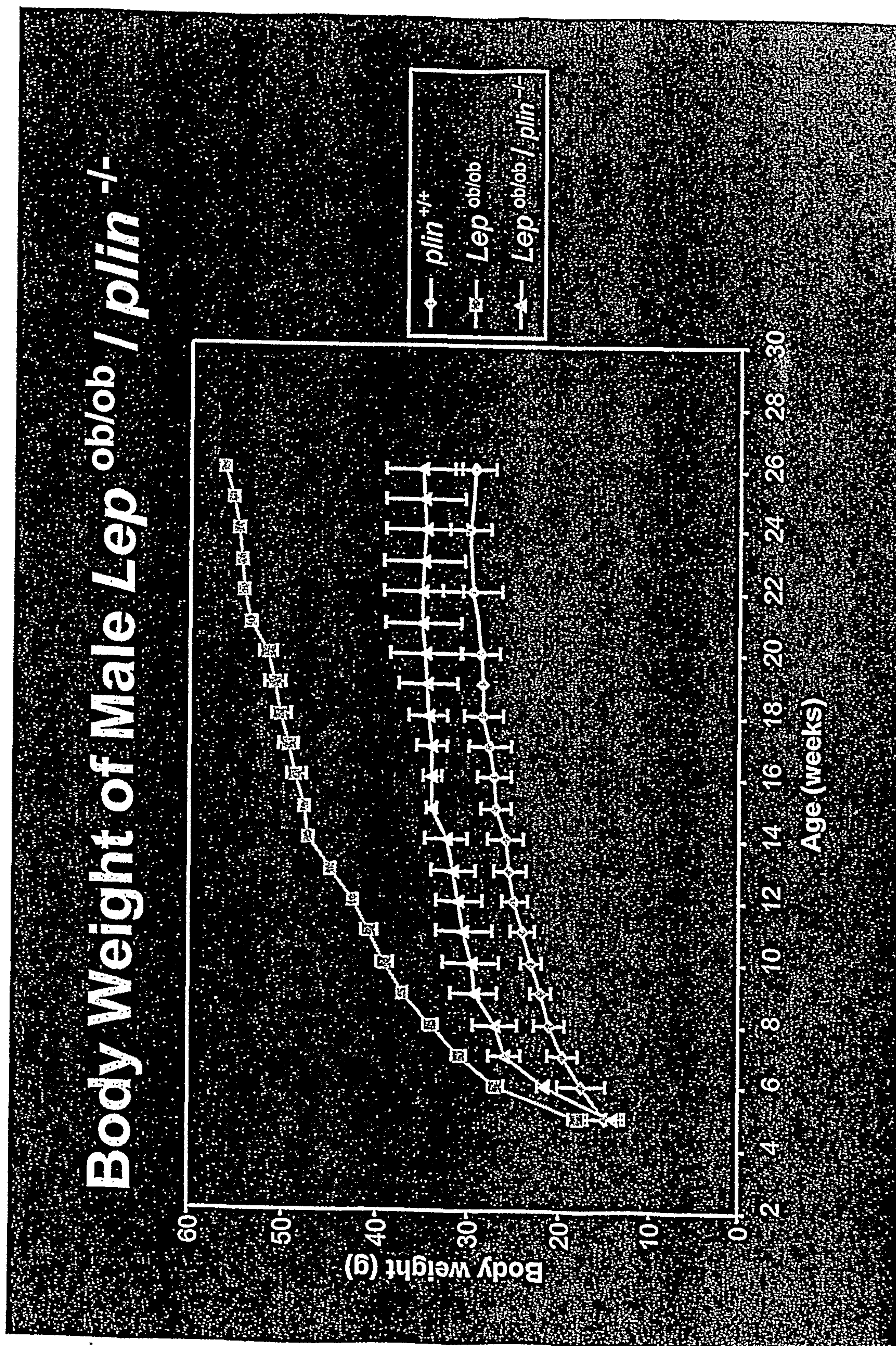


FIG. 8A

Body Weight of Female *Lep^{ob/ob} / plin^{-/-}*

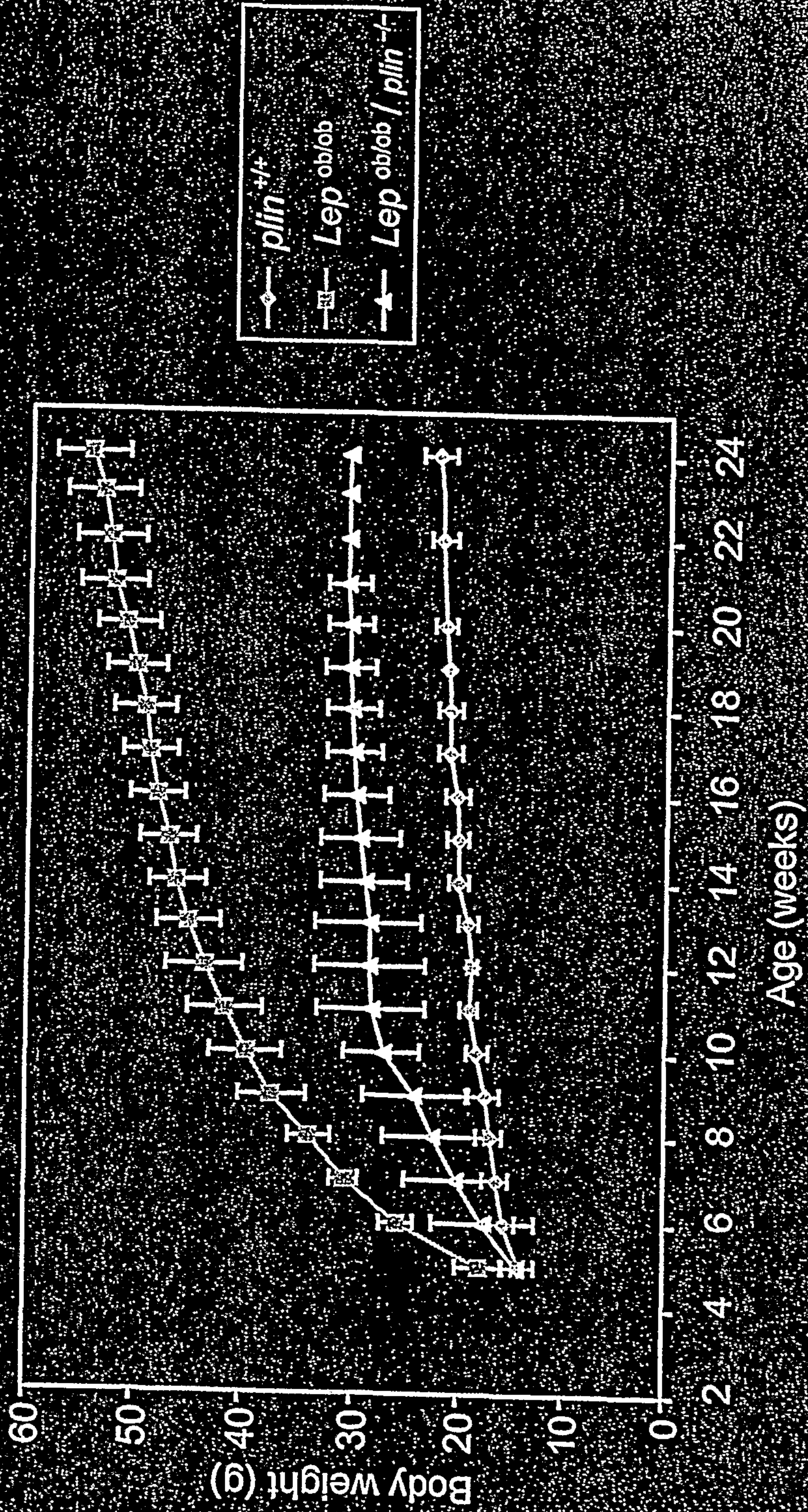


FIG. 8B

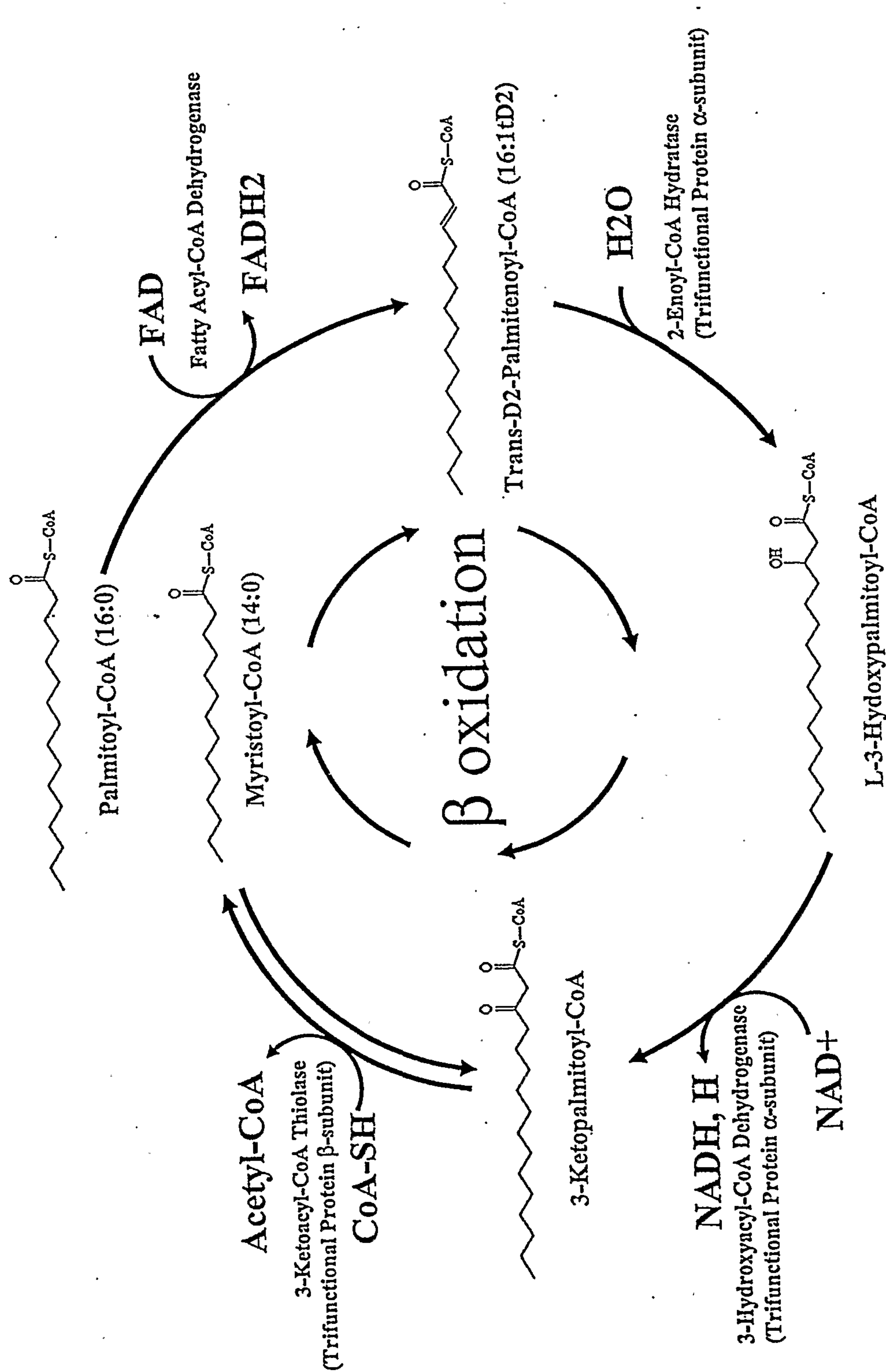


FIG. 9

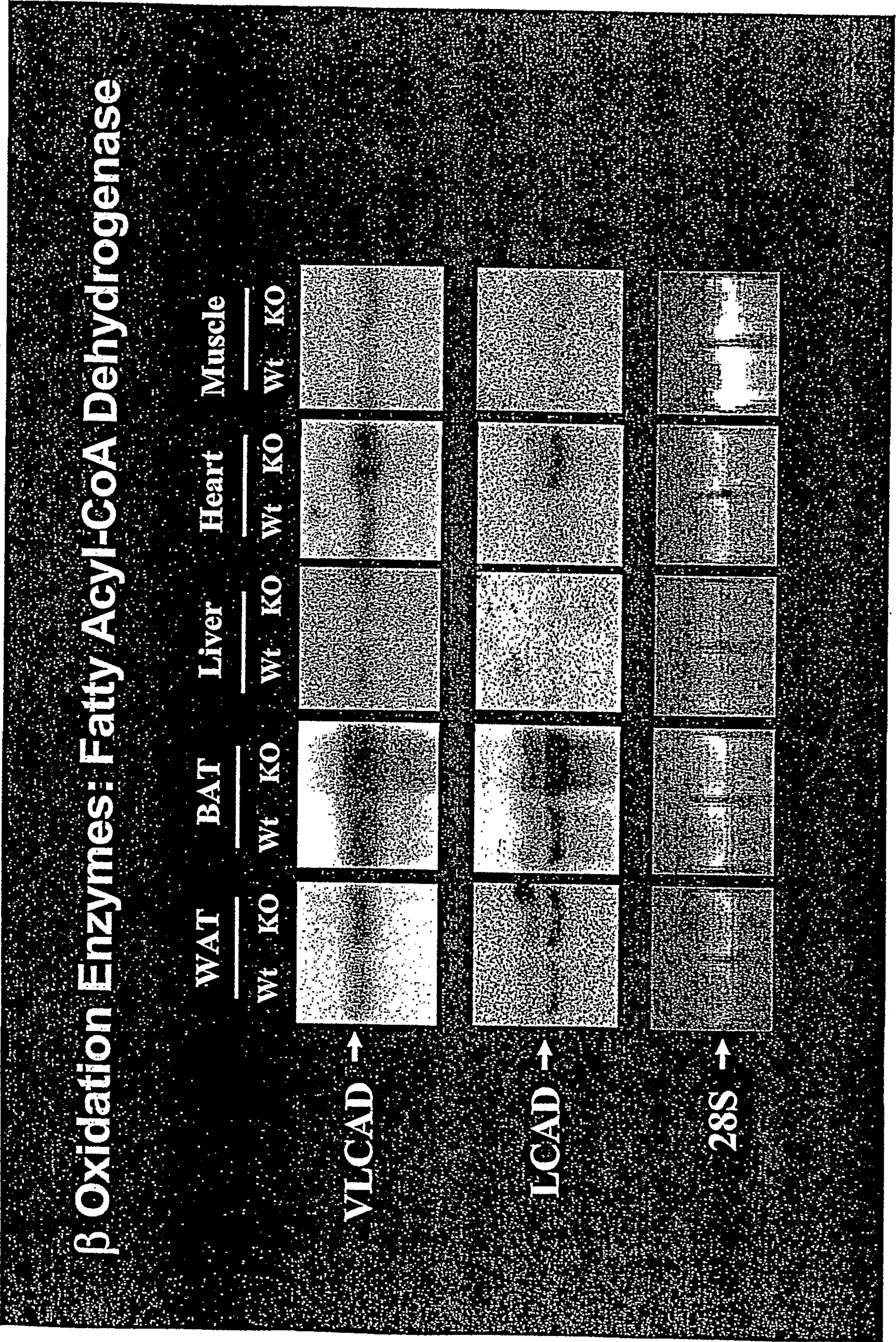


FIG. 10

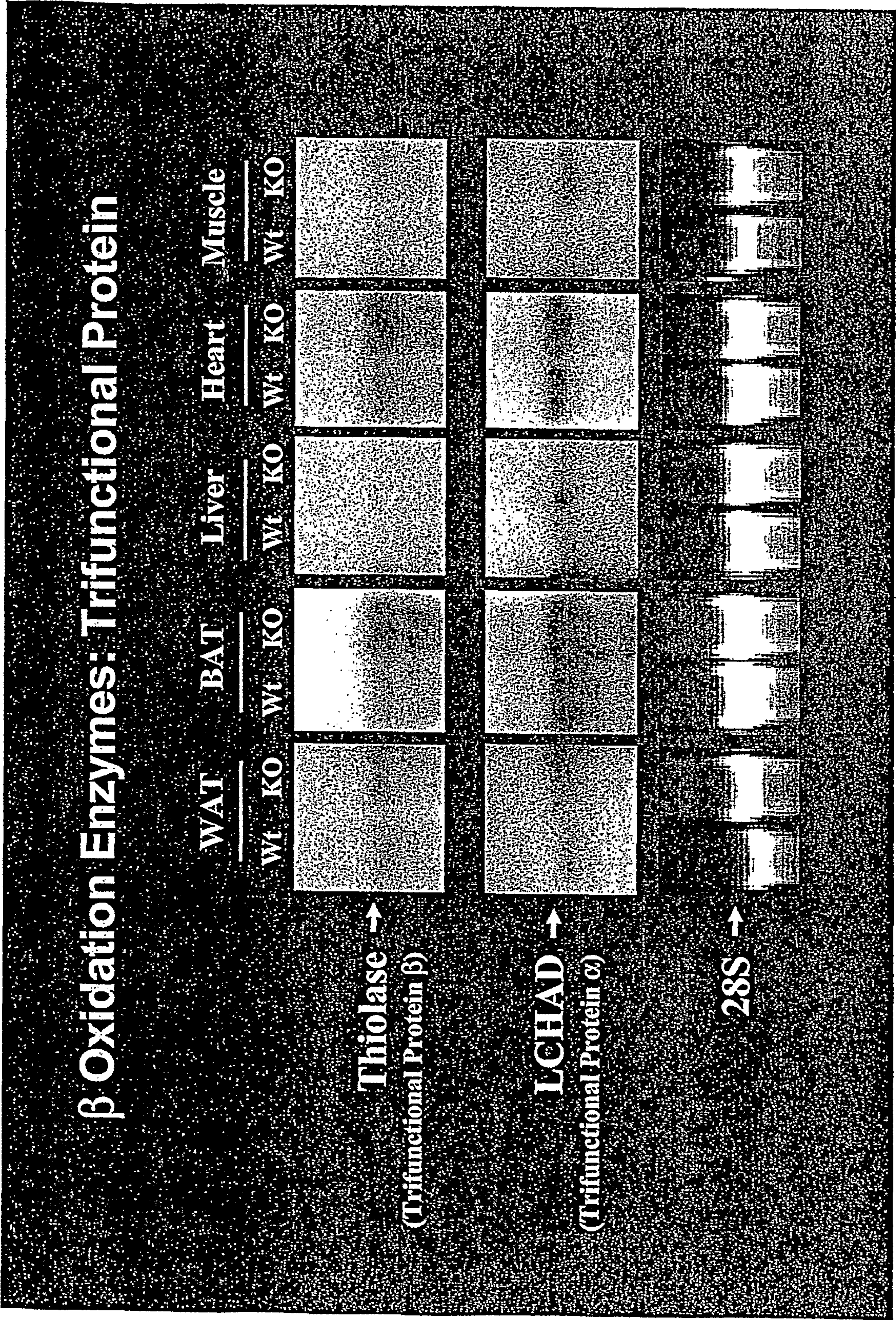


FIG. 11

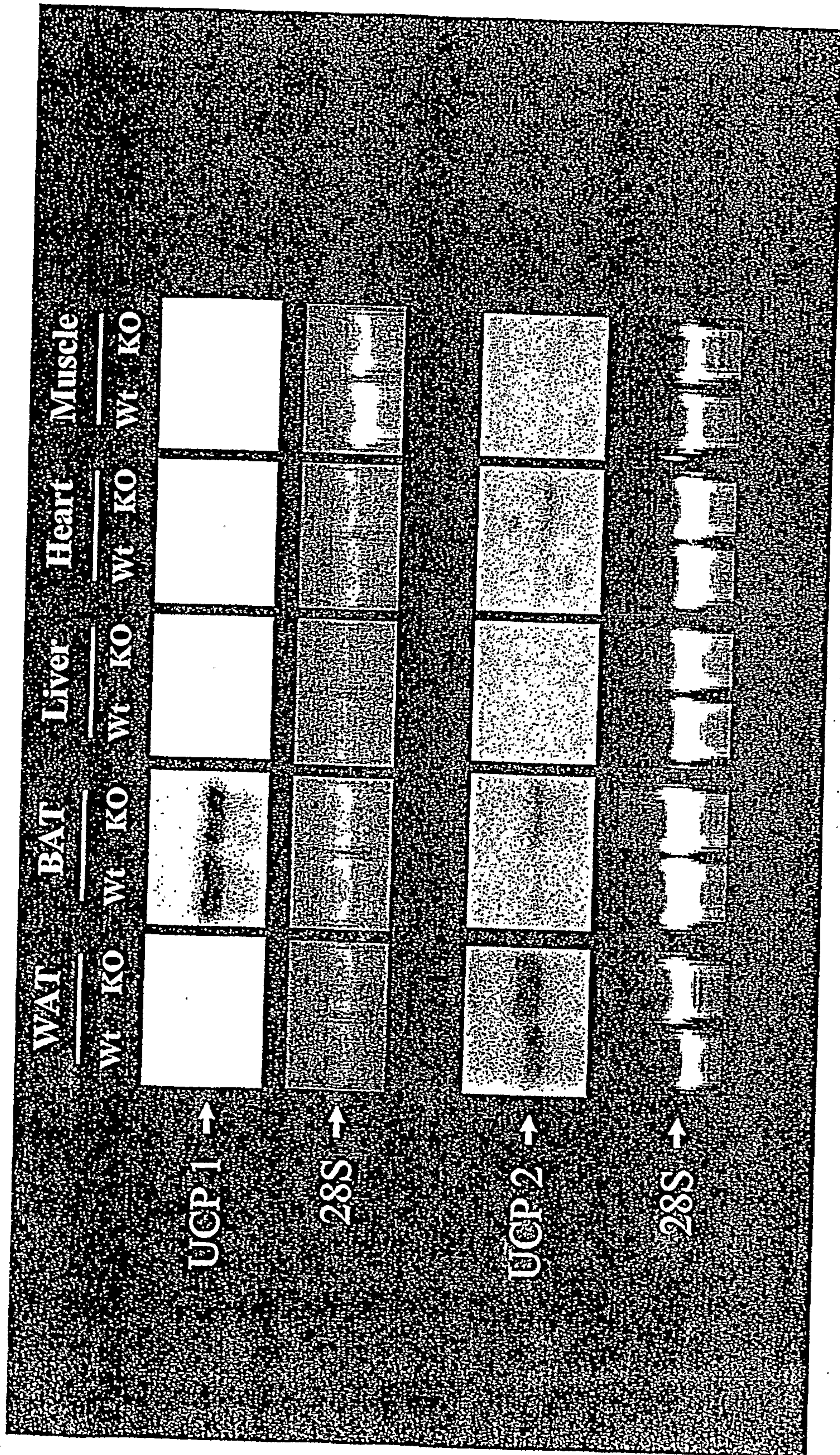


FIG. 12

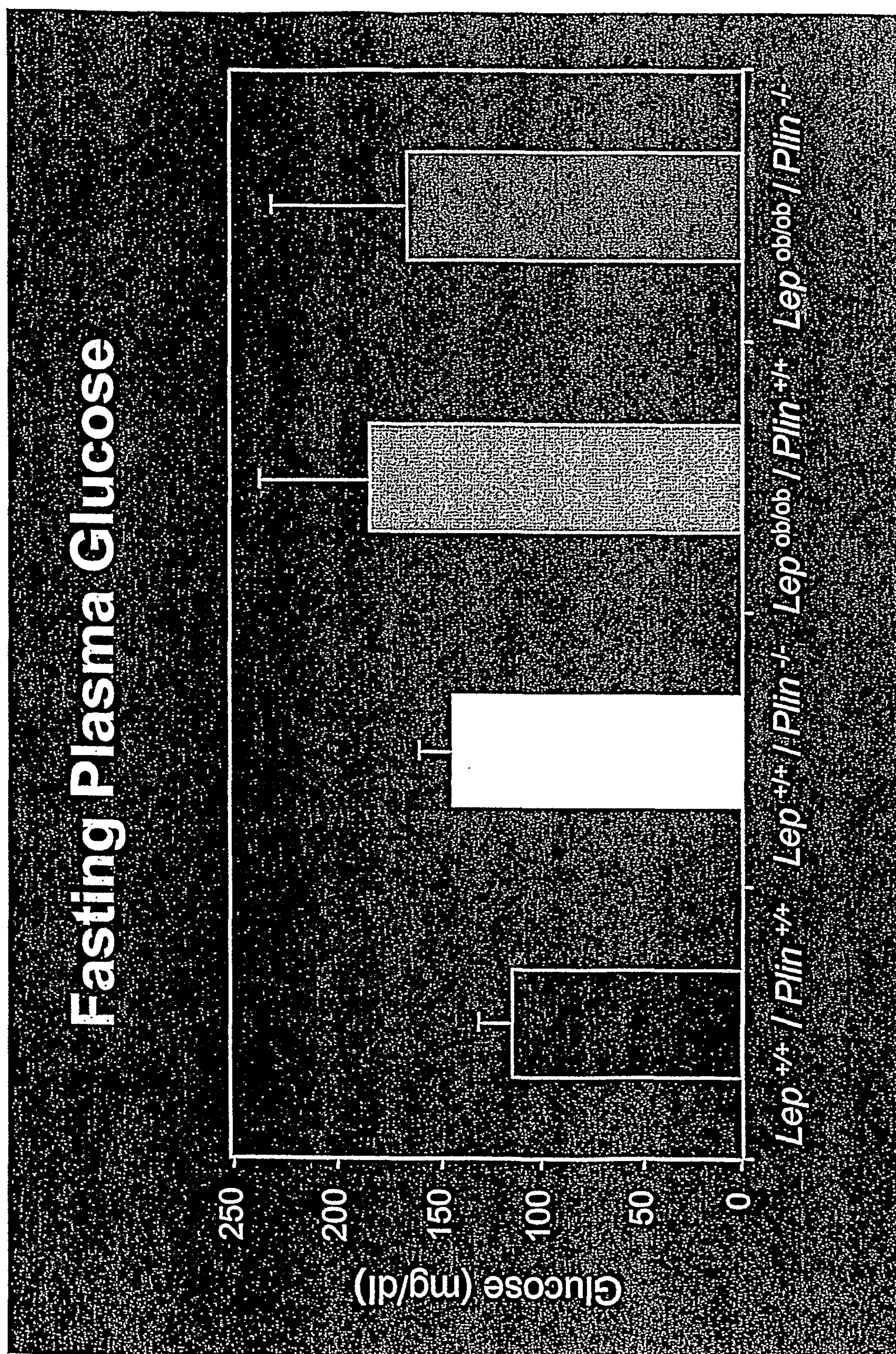


FIG. 13A

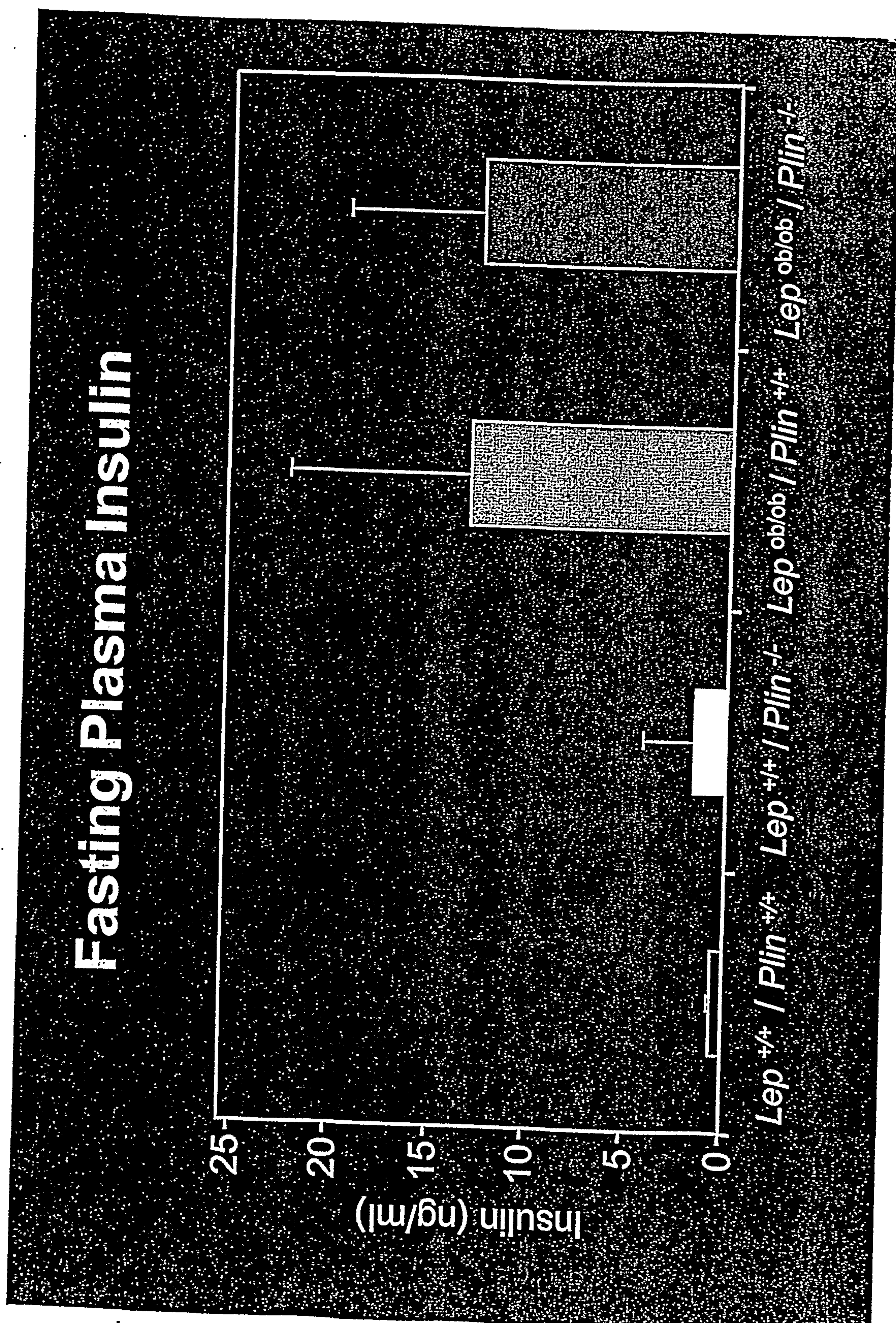


FIG. 13B

GTT (1.5 g/kg) of *Lep ob/ob* / *Plin* ^{-/-}

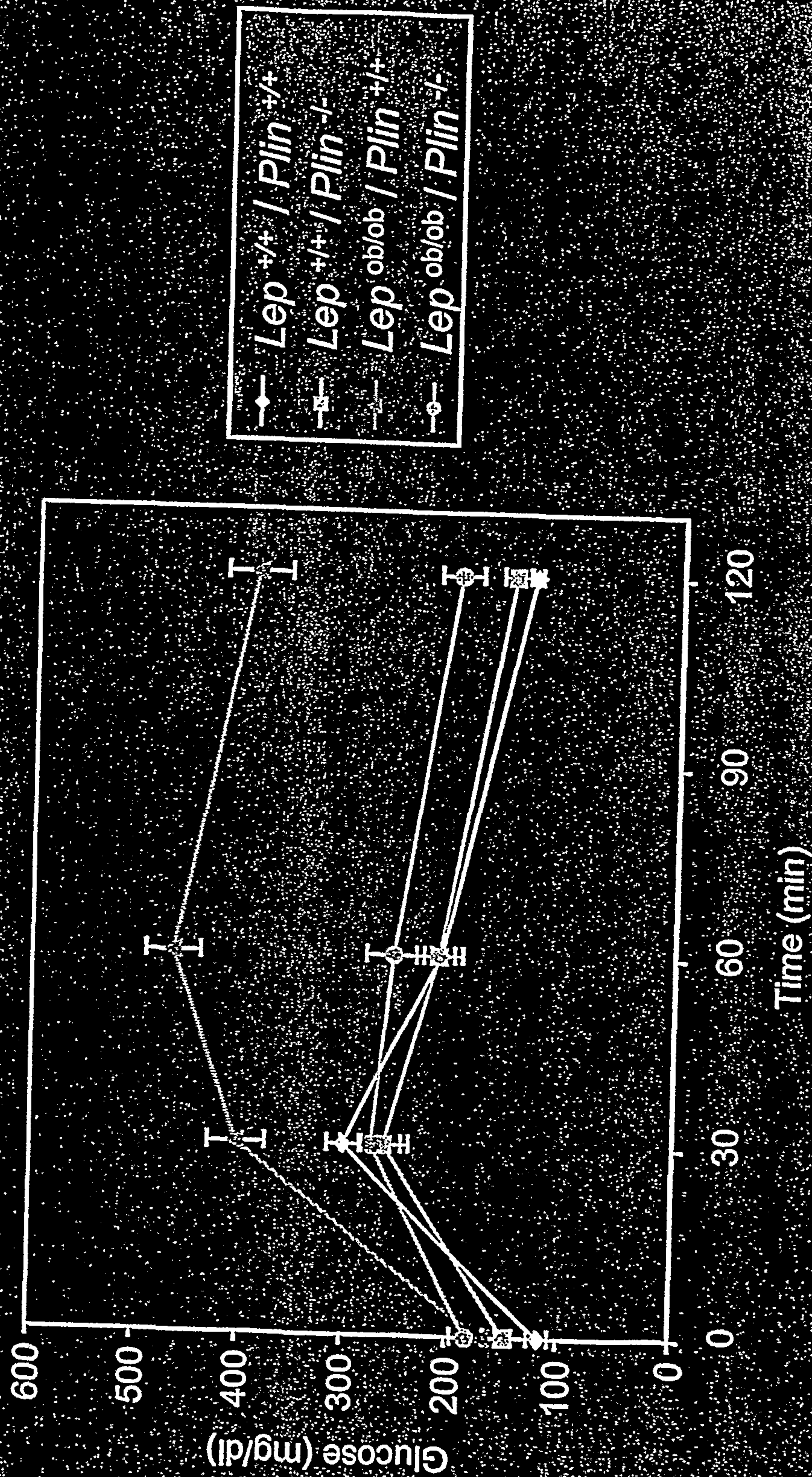


FIG. 14A

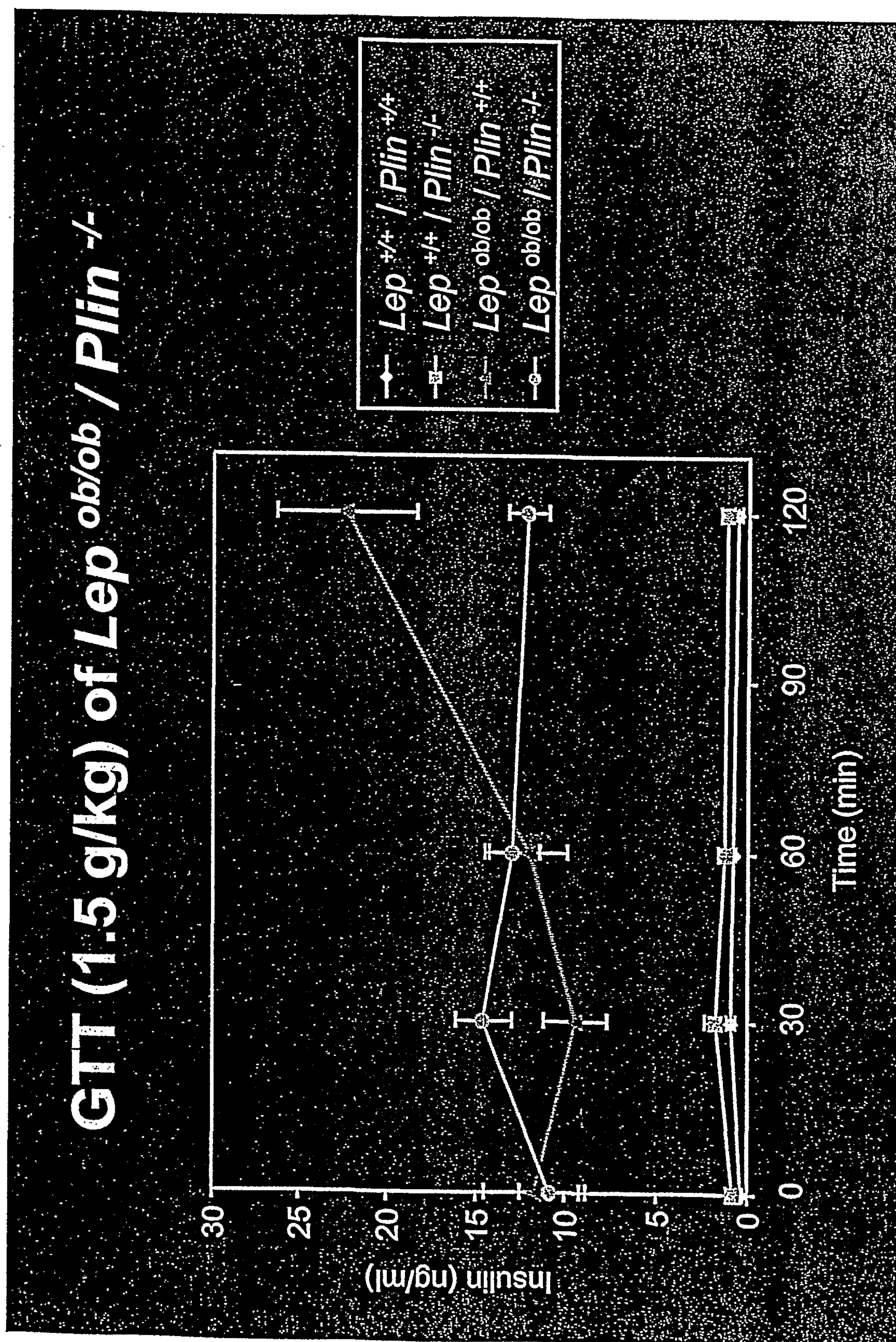


FIG. 14B

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US01/31400

A. CLASSIFICATION OF SUBJECT MATTER

IPC(7) :A61K 38/00

US CL :514/2

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 514/2

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

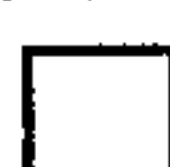
Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	US 5,585,462 A (LONDOS et al.) 17 December 1996 (17.12.96), see the entire document.	1-31
A	US 5,739,009 A (HILLMAN et al.) 14 April 1998 (14.04.98), see the entire document.	1-31



Further documents are listed in the continuation of Box C.



See patent family annex.

* Special categories of cited documents:		"I"	later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"A"	document defining the general state of the art which is not considered to be of particular relevance	"X"	document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"E"	earlier document published on or after the international filing date	"Y"	document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"L"	document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)	"G"	document member of the same patent family
"O"	document referring to an oral disclosure, use, exhibition or other means		
"P"	document published prior to the international filing date but later than the priority date claimed		

Date of the actual completion of the international search

12 NOVEMBER 2001

Date of mailing of the international search report

18 JAN 2002

Name and mailing address of the ISA/US
Commissioner of Patents and Trademarks
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Washington, D.C. 20231

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