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(54) Title: METHODS FOR TREATING DIABETES

(57) Abstract

This invention describes methods of treating diabetes in obese type II diabetic patients. Specifically, methods of treating obese type II diabetics with varying levels of endogenous leptin is claimed.

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METHODS FOR TREATING DIABETES

Diabetes mellitus is a metabolic disorder characterized by the failure of body tissues to store carbohydrates at the normal rate. Resistance to the action of insulin is the most important factor to Type II diabetes. When this resistance exceeds the capacity of the beta cells to produce insulin, a person becomes diabetic. During the last 70 years people suffering from diabetes have been greatly aided by receiving controlled amounts of insulin.

Obesity, particularly upper body obesity, is often associated with non-insulin-dependent diabetes mellitus (NIDDM). These so called Type II diabetics do not have an absolute requirement for insulin as their beta cells are able to secrete insulin, albeit often at diminished levels. In addition such patients are often obese and may demonstrate an inability to respond to insulin.

It is well known that a regimen of diet and exercise leading to weight loss is the best approach for treating obese type II diabetics. Unfortunately, these regimens are usually unsuccessful. Failure to loss weight may be due to genetically inherited factors that contribute to increased appetite, a preference for high calorie foods, reduced physical activity, and an increased lipogenic metabolism. People inheriting such genetic predispositions are prone to obesity and often become type II diabetics, regardless of their efforts to combat the condition. The *ob /ob* mouse is a model of obesity and diabetes that is known to carry an autosomal recessive trait linked to a mutation in the sixth chromosome. Recently, Yiying Zhang and co-workers published the positional cloning of the mouse gene (*ob*) linked with this condition. Yiying Zhang et al. Nature 372: 425-32 (1994). This report disclosed a gene coding for a 167 amino acid protein (hereinafter leptin) with a 21 amino acid signal peptide that is exclusively expressed in adipose tissue.

Circulating levels of leptin in obese individuals have been shown to vary widely. Consequently, it is now believed that a subpopulation of obese type II diabetics are particularly amenable to treatment with leptin. Pharmacological agents which are biologically active and mimic the activity of leptin are therefore useful for treating obese type II diabetics, particularly those with abnormally high or low levels of circulating leptin.

One aspect of the present invention is a method of treating or preventing diabetes which comprises administering to an obese type II diabetic an effective amount of leptin, leptin mimetic, or a pharmaceutically acceptable salt thereof. In a preferred embodiment, the invention includes methods for treating obese type II diabetics having low endogenous levels of leptin.

Obesity refers to a condition in which the individual has a body mass index of greater than 27 kilograms per square meter.

Leptin refers to the protein produced from the obesity gene following transcription and deletions of introns, translation to a protein and processing to the mature protein with secretory signal peptide removed, e.g., from the N-terminal valine-proline to the C-terminal cysteine of the mature protein. Mouse and human leptin protein sequences are published in Zhang *et al.* Nature 372: 425-32 (1994). The rat leptin sequence is published in Murakami et al., Biochemical and Biophysical Research Comm. 209(3): 944-52 (1995). In human, murine and rat leptin the Cys associated with di-sulfide formation is positions 96 and 145. However, particularly with murine and human leptin, a desGln(28) variant has been observed. Hence, the Cys residues associated with di-sulfide bond formation may be at positions 95 or 96 and at position 145 or 146. Leptin may also be referred to throughout this specification as obesity protein, OB, or *ob* gene product. Leptin therefore includes SEQ ID NOs:1-6.

Those skilled in the art will recognize that certain amino acids are prone to rearrangement. For example, Asp may rearrange to aspartimide and isoasparigine as described in I. Schön, et al., International Journal of Peptide and Protein Research, 14:485-94 (1979) and references cited therein. These rearrangement derivatives are included within the scope of the present invention. Unless otherwise indicated the amino acids are in the L configuration.

Preferred forms of leptin useful in the presently claimed method are the native sequences. The use of human leptin is more preferred. Most preferred leptins useful in the present method include proteins of SEQ ID NOS:1-6.

Murine Leptin

SEQ ID NO: 1

```
Val Pro Ile Gln Lys Val Gln Asp Asp Thr Lys Thr Leu Ile Lys Thr
1               5                  10                  15

Ile Val Thr Arg Ile Asn Asp Ile Ser His Thr Xaa Ser Val Ser Ser
            20                  25                  30

Lys Gln Lys Val Thr Gly Leu Asp Phe Ile Pro Gly Leu His Pro Ile
        35                  40                  45

Leu Thr Leu Ser Lys Met Asp Gln Thr Leu Ala Val Tyr Gln Gln Ile
    50                  55                  60

Leu Thr Ser Met Pro Ser Arg Asn Val Ile Gln Ile Ser Asn Asp Leu
65                  70                  75                  80

Glu Asn Leu Arg Asp Leu Leu His Val Leu Ala Phe Ser Lys Ser Cys
                85                  90                  95

His Leu Pro Gln Ala Ser Gly Leu Glu Thr Leu Glu Ser Leu Gly Gly
            100                 105                 110

Val Leu Glu Ala Ser Gly Tyr Ser Thr Glu Val Val Ala Leu Ser Arg
        115                 120                 125

Leu Gln Gly Ser Leu Gln Asp Met Leu Gln Gln Leu Asp Leu Ser Pro
    130                 135                 140

Gly Cys
145
```

wherein:

Xaa at position 28 is Gln or absent.

Porcine Leptin

SEQ ID NO: 2

```
Val Pro Ile Trp Arg Val Gln Asp Asp Thr Lys Thr Leu Ile Lys Thr
 1               5                  10                  15

Ile Val Thr Arg Ile Ser Asp Ile Ser His Met Gln Ser Val Ser Ser
            20                  25                  30

Lys Gln Arg Val Thr Gly Leu Asp Phe Ile Pro Gly Leu His Pro Val
        35                  40                  45

Leu Ser Leu Ser Lys Met Asp Gln Thr Leu Ala Ile Tyr Gln Gln Ile
    50                  55                  60

Leu Thr Ser Leu Pro Ser Arg Asn Val Ile Gln Ile Ser Asn Asp Leu
65                  70                  75                  80

Glu Asn Leu Arg Asp Leu Leu His Leu Leu Ala Ser Ser Lys Ser Cys
                85                  90                  95

Pro Leu Pro Gln Ala Arg Ala Leu Glu Thr Leu Glu Ser Leu Gly Gly
            100                 105                 110

Val Leu Glu Ala Ser Leu Tyr Ser Thr Glu Val Val Ala Leu Ser Arg
        115                 120                 125

Leu Gln Gly Ala Leu Gln Asp Met Leu Arg Gln Leu Asp Leu Ser Pro
    130                 135                 140

Gly Cys
145
```

Bovine Leptin

SEQ ID NO: 3

```
Val Pro Ile Cys Lys Val Gln Asp Asp Thr Lys Thr Leu Ile Lys Thr
1               5                  10                  15

Ile Val Thr Arg Ile Asn Asp Ile Ser His Thr Xaa Ser Val Ser Ser
            20                  25                  30

Lys Gln Arg Val Thr Gly Leu Asp Phe Ile Pro Gly Leu His Pro Leu
        35                  40                  45

Leu Ser Leu Ser Lys Met Asp Gln Thr Leu Ala Ile Tyr Gln Gln Ile
    50                  55                  60

Leu Thr Ser Leu Pro Ser Arg Asn Val Val Gln Ile Ser Asn Asp Leu
65                  70                  75                  80

Glu Asn Leu Arg Asp Leu Leu His Leu Leu Ala Ala Ser Lys Ser Cys
                85                  90                  95
```

```
Pro Leu Pro Gln Val Arg Ala Leu Glu Ser Leu Glu Ser Leu Gly Val
            100                 105                 110

Val Leu Glu Ala Ser Leu Tyr Ser Thr Glu Val Val Ala Leu Ser Arg
        115                 120                 125

Leu Gln Gly Ser Leu Gln Asp Met Leu Arg Gln Leu Asp Leu Ser Pro
    130                 135                 140

Gly Cys
145
```

wherein Xaa at position 28 is Gln or absent.

Human Leptin

SEQ ID NO: 4

```
Val Pro Ile Gln Lys Val Gln Asp Asp Thr Lys Thr Leu Ile Lys Thr
1               5                   10                  15

Ile Val Thr Arg Ile Asn Asp Ile Ser His Xaa Xaa Ser Val Ser Ser
            20                  25                  30

Lys Gln Lys Val Thr Gly Leu Asp Phe Ile Pro Gly Leu His Pro Ile
        35                  40                  45

Leu Thr Leu Ser Lys Met Asp Gln Thr Leu Ala Val Tyr Gln Gln Ile
    50                  55                  60

Leu Thr Ser Met Pro Ser Arg Asn Val Ile Gln Ile Ser Asn Asp Leu
65                  70                  75                  80

Glu Asn Leu Arg Asp Leu Leu His Val Leu Ala Phe Ser Lys Ser Cys
                85                  90                  95

His Leu Pro Trp Ala Ser Gly Leu Glu Thr Leu Asp Ser Leu Gly Gly
            100                 105                 110

Val Leu Glu Ala Ser Gly Tyr Ser Thr Glu Val Val Ala Leu Ser Arg
        115                 120                 125

Leu Gln Gly Ser Leu Gln Asp Met Leu Trp Gln Leu Asp Leu Ser Pro
    130                 135                 140

145
Gly Cys
```

wherein:

Xaa at position 27 is Thr or Ala; and

Xaa at position 28 is Gln or absent.

Rhesus Leptin

SEQ ID NO:5

```
Val Pro Ile Gln Lys Val Gln Ser Asp Thr Lys Thr Leu Ile Lys
1               5                  10                  15

Thr Ile Val Thr Arg Ile Asn Asp Ile Ser His Thr Gln Ser Val
            20                  25                  30

Ser Ser Lys Gln Arg Val Thr Gly Leu Asp Phe Ile Pro Gly Leu
        35                  40                  45

His Pro Val Leu Thr Leu Ser Gln Met Asp Gln Thr Leu Ala Ile
    50                  55                  60

Tyr Gln Gln Ile Leu Ile Asn Leu Pro Ser Arg Asn Val Ile Gln
65                  70                  75

Ile Ser Asn Asp Leu Glu Asn Leu Arg Asp Leu Leu His Leu Leu
            80                  85                  90

Ala Phe Ser Lys Ser Cys His Leu Pro Leu Ala Ser Gly Leu Glu
        95                  100                 105

Thr Leu Glu Ser Leu Gly Asp Val Leu Glu Ala Ser Leu Tyr Ser
    110                 115                 120

Thr Glu Val Val Ala Leu Ser Arg Leu Gln Gly Ser Leu Gln Asp
125                 130                 135

Met Leu Trp Gln Leu Asp Leu Ser Pro Gly Cys
            140                 145
```

Rat Leptin

SEQ ID NO:6

```
Val Pro Ile His Lys Val Gln Asp Asp Thr Lys Thr Leu Ile Lys Thr
1               5                  10                  15

Ile Val Thr Arg Ile Asn Asp Ile Ser His Thr Gln Ser Val Ser Ala
            20                  25                  30

Arg Gln Arg Val Thr Gly Leu Asp Phe Ile Pro Gly Leu His Pro Ile
        35                  40                  45

Leu Ser Leu Ser Lys Met Asp Gln Thr Leu Ala Val Tyr Gln Gln Ile
    50                  55                  60

Leu Thr Ser Leu Pro Ser Gln Asn Val Leu Gln Ile Ala His Asp Leu
65                  70                  75                  80

Glu Asn Leu Arg Asp Leu Leu His Leu Leu Ala Phe Ser Lys Ser Cys
                85                  90                  95

Ser Leu Pro Gln Thr Arg Gly Leu Gln Lys Pro Glu Ser Leu Asp Gly
            100                 105                 110

Val Leu Glu Ala Ser Leu Tyr Ser Thr Glu Val Val Ala Leu Ser Arg
```

115 120 125

Leu Gln Gly Ser Leu Gln Asp Ile Leu Gln Gln Leu Asp Leu Ser Pro
130 135 140

Glu Cys
145

In addition to the above identified protein sequences, it is frequently considered expeditious to prepare such anti diabetic proteins with a one or two amino acid leader sequence, especially with a methionine containing leader. Two frequently employed leaders are Met-Arg and Met-Asp. Such proteins may be identified infra as Met-Arg-leptin or Met-Asp-leptin or may be identified by Met-Arg-SEQ ID NO:X, where X is 1 to 6.

Leptin mimetics and fragments are also useful in the methods of the present invention. Leptin mimetics are generally defined by Formula (I) (SEQ ID NO:7) as follows:

Val Pro Ile Gln Lys Val Gln Asp Asp Thr Lys Thr Leu Ile Lys Thr
1 5 10 15

Ile Val Thr Arg Ile Xaa Asp Ile Ser His Xaa Xaa Ser Val Ser Ser
20 25 30

Lys Gln Lys Val Thr Gly Leu Asp Phe Ile Pro Gly Leu His Pro Ile
35 40 45

Leu Thr Leu Ser Lys Xaa Asp Gln Thr Leu Ala Val Tyr Gln Gln Ile
50 55 60

Leu Thr Ser Xaa Pro Ser Arg Xaa Val Ile Gln Ile Xaa Asn Asp Leu
65 70 75 80

Glu Asn Leu Arg Asp Leu Leu His Val Leu Ala Phe Ser Lys Ser Cys
85 90 95

His Leu Pro Trp Ala Ser Gly Leu Glu Thr Leu Asp Ser Leu Gly Gly
100 105 110

Val Leu Glu Ala Ser Xaa Tyr Ser Thr Glu Val Val Ala Leu Ser Arg
115 120 125

Leu Gln Gly Ser Leu Gln Asp Met Leu Trp Gln Leu Asp Leu Ser Pro
130 135 140

145
Gly Cys

wherein:

Xaa at position 22 is Asn, Asp or Glu;

Xaa at position 27 is Thr or Ala;

Xaa at position 28 is Gln, Glu, or absent;

Xaa at position 54 is Met or Ala;

Xaa at position 68 is Met or Leu;

Xaa at position 72 Asn, Asp or Glu;

Xaa at position 77 is Ser or Ala;

Xaa at position 118 is Gly or Leu;

said protein having at least one substitution selected from the group consisting of:

His at position 97 is replaced with Ser or Pro;

Trp at position 100 is replaced with Gln, Ala or Leu;

Ala at position 101 is replaced with Thr or Val;

Ser at position 102 is replaced with Arg;

Gly at position 103 is replaced with Ala;

Glu at position 105 is replaced with Gln;

Thr at position 106 is replaced with Lys or Ser;

Leu at position 107 is replaced with Pro;

Asp at position 108 is replaced with Glu; or

Gly at position 111 is replaced with Asp.

Preferably, leptin mimetics are those of Formula (II) (SEQ ID NO:8) as follows:

```
Val Pro Ile Gln Lys Val Gln Asp Asp Thr Lys Thr Leu Ile Lys Thr
1               5                  10                  15

Ile Val Thr Arg Ile Asn Asp Ile Ser His Xaa Gln Ser Val Ser Ser
            20                  25                  30

Lys Gln Lys Val Thr Gly Leu Asp Phe Ile Pro Gly Leu His Pro Ile
        35                  40                  45

Leu Thr Leu Ser Lys Met Asp Gln Thr Leu Ala Val Tyr Gln Gln Ile
    50                  55                  60

Leu Thr Ser Met Pro Ser Arg Asn Val Ile Gln Ile Xaa Asn Asp Leu
65                  70                  75                  80

Glu Asn Leu Arg Asp Leu Leu His Val Leu Ala Phe Ser Lys Ser Cys
                85                  90                  95

His Leu Pro Trp Ala Ser Gly Leu Glu Thr Leu Asp Ser Leu Gly Gly
```

100 105 110

Val Leu Glu Ala Ser Xaa Tyr Ser Thr Glu Val Val Ala Leu Ser Arg
115 120 125

Leu Gln Gly Ser Leu Gln Asp Met Leu Trp Gln Leu Asp Leu Ser Pro
130 135 140

145

Gly Cys

wherein:

Xaa at position 27 is Thr or Ala;

Xaa at position 77 is Ser or Ala;

Xaa at position 118 is Gly or Leu;

said protein having at least one substitution, preferably having one to five substitutions and, most preferably, one to two substitutions selected from the group consisting of:

His at position 97 is replaced with Ser;

Trp at position 100 is replaced with Gln;

Ala at position 101 is replaced with Thr;

Glu at position 105 is replaced with Gln;

Thr at position 106 is replaced with Lys;

Leu at position 107 is replaced with Pro;

Asp at position 108 is replaced with Glu; or

Gly at position 111 is replaced with Asp.

Examples of preferred proteins of the present invention include proteins of SEQ ID NO:8, wherein Xaa at position 27 is Thr; Xaa at position 77 is Ser; Xaa at position 118 is Gly; and the amino acid residues at positions 97, 100, 101, 105, 106, 107, 108, and 111 are as follows in Table I. The native human sequence is provided as a comparison to the proteins employed in the methods of the present invention.

Table I

	Amino Acid Position							
Compound	97	100	101	105	106	107	108	111

native human	His	Trp	Ala	Glu	Thr	Leu	Asp	Gly
1	Ser	Trp	Ala	Glu	Thr	Leu	Asp	Gly
2	His	Gln	Ala	Glu	Thr	Leu	Asp	Gly
3	His	Trp	Thr	Glu	Thr	Leu	Asp	Gly
4	His	Trp	Ala	Gln	Thr	Leu	Asp	Gly
5	His	Trp	Ala	Glu	Lys	Leu	Asp	Gly
6	His	Trp	Ala	Glu	Thr	Pro	Asp	Gly
7	His	Trp	Ala	Glu	Thr	Leu	Glu	Gly
8	His	Trp	Ala	Glu	Thr	Leu	Asp	Asp
9	Ser	Gln	Ala	Glu	Thr	Leu	Asp	Gly
10	Ser	Trp	Thr	Glu	Thr	Leu	Asp	Gly
11	Ser	Trp	Ala	Gln	Thr	Leu	Asp	Gly
12	Ser	Trp	Ala	Glu	Lys	Leu	Asp	Gly
13	Ser	Trp	Ala	Glu	Thr	Pro	Asp	Gly
14	Ser	Trp	Ala	Glu	Thr	Leu	Glu	Gly
15	Ser	Trp	Ala	Glu	Thr	Leu	Asp	Asp
16	His	Gln	Thr	Glu	Thr	Leu	Asp	Gly
17	His	Gln	Ala	Gln	Thr	Leu	Asp	Gly
18	His	Gln	Ala	Glu	Lys	Leu	Asp	Gly
19	His	Gln	Ala	Glu	Thr	Pro	Asp	Gly
20	His	Gln	Ala	Glu	Thr	Leu	Glu	Gly
21	His	Gln	Ala	Glu	Thr	Leu	Asp	Asp
22	His	Trp	Thr	Gln	Thr	Leu	Asp	Gly
23	His	Trp	Thr	Glu	Lys	Leu	Asp	Gly
24	His	Trp	Thr	Glu	Thr	Pro	Asp	Gly
25	His	Trp	Thr	Glu	Thr	Leu	Glu	Gly
26	His	Trp	Thr	Glu	Thr	Leu	Asp	Asp
27	His	Trp	Ala	Gln	Lys	Leu	Asp	Gly
28	His	Trp	Ala	Gln	Thr	Pro	Asp	Gly
29	His	Trp	Ala	Gln	Thr	Leu	Glu	Gly
30	His	Trp	Ala	Gln	Thr	Leu	Asp	Asp
31	His	Trp	Ala	Glu	Lys	Pro	Asp	Gly
32	His	Trp	Ala	Glu	Lys	Leu	Glu	Gly
33	His	Trp	Ala	Glu	Lys	Leu	Asp	Asp
34	His	Trp	Ala	Glu	Thr	Pro	Glu	Gly
35	His	Trp	Ala	Glu	Thr	Pro	Asp	Asp

36	His	Trp	Ala	Glu	Thr	Leu	Glu	Asp
37	Ser	Gln	Thr	Glu	Thr	Leu	Asp	Gly
38	Ser	Gln	Ala	Gln	Thr	Leu	Asp	Gly
39	Ser	Gln	Ala	Glu	Lys	Leu	Asp	Gly
40	Ser	Gln	Ala	Glu	Thr	Pro	Asp	Gly
41	Ser	Gln	Ala	Glu	Thr	Leu	Glu	Gly
42	Ser	Gln	Ala	Glu	Thr	Leu	Asp	Asp
43	Ser	Trp	Thr	Gln	Thr	Leu	Asp	Gly
44	Ser	Trp	Thr	Glu	Lys	Leu	Asp	Gly
45	Ser	Trp	Thr	Glu	Thr	Pro	Asp	Gly
46	Ser	Trp	Thr	Glu	Thr	Leu	Glu	Gly
47	Ser	Trp	Thr	Glu	Thr	Leu	Asp	Asp
48	Ser	Trp	Ala	Gln	Lys	Leu	Asp	Gly
49	Ser	Trp	Ala	Gln	Thr	Pro	Asp	Gly
50	Ser	Trp	Ala	Gln	Thr	Leu	Glu	Gly
51	Ser	Trp	Ala	Gln	Thr	Leu	Asp	Asp
52	Ser	Trp	Ala	Glu	Lys	Pro	Asp	Gly
53	Ser	Trp	Ala	Glu	Lys	Leu	Glu	Gly
54	Ser	Trp	Ala	Glu	Lys	Leu	Asp	Asp
55	Ser	Trp	Ala	Glu	Thr	Pro	Glu	Gly
56	Ser	Trp	Ala	Glu	Thr	Pro	Asp	Asp
57	Ser	Trp	Ala	Glu	Thr	Leu	Glu	Asp
58	His	Gln	Thr	Gln	Thr	Leu	Asp	Gly
59	His	Gln	Thr	Glu	Lys	Leu	Asp	Gly
60	His	Gln	Thr	Glu	Thr	Pro	Asp	Gly
61	His	Gln	Thr	Glu	Thr	Leu	Glu	Gly
62	His	Gln	Thr	Glu	Thr	Leu	Asp	Asp
63	His	Gln	Ala	Gln	Lys	Leu	Asp	Gly
64	His	Gln	Ala	Gln	Thr	Pro	Asp	Gly
65	His	Gln	Ala	Gln	Thr	Leu	Glu	Gly
66	His	Gln	Ala	Gln	Thr	Leu	Asp	Asp
67	His	Gln	Ala	Glu	Lys	Pro	Asp	Gly
68	His	Gln	Ala	Glu	Lys	Leu	Glu	Gly
69	His	Gln	Ala	Glu	Lys	Leu	Asp	Asp
70	His	Gln	Ala	Glu	Thr	Pro	Glu	Gly
71	His	Gln	Ala	Glu	Thr	Pro	Asp	Asp

72	His	Gln	Ala	Glu	Thr	Leu	Glu	Asp
73	His	Trp	Thr	Gln	Lys	Leu	Asp	Gly
74	His	Trp	Thr	Gln	Thr	Pro	Asp	Gly
75	His	Trp	Thr	Gln	Thr	Leu	Glu	Gly
76	His	Trp	Thr	Gln	Thr	Leu	Asp	Asp
77	His	Trp	Thr	Glu	Lys	Pro	Asp	Gly
78	His	Trp	Thr	Glu	Lys	Leu	Glu	Gly
79	His	Trp	Thr	Glu	Lys	Leu	Asp	Asp
80	His	Trp	Thr	Glu	Thr	Pro	Glu	Gly
81	His	Trp	Thr	Glu	Thr	Pro	Asp	Asp
82	His	Trp	Thr	Glu	Thr	Leu	Glu	Asp
83	His	Trp	Ala	Gln	Lys	Pro	Asp	Gly
84	His	Trp	Ala	Gln	Lys	Leu	Glu	Gly
85	His	Trp	Ala	Gln	Lys	Leu	Asp	Asp
86	His	Trp	Ala	Gln	Thr	Pro	Glu	Gly
87	His	Trp	Ala	Gln	Thr	Pro	Asp	Asp
88	His	Trp	Ala	Gln	Thr	Leu	Glu	Asp
89	His	Trp	Ala	Glu	Lys	Pro	Glu	Gly
90	His	Trp	Ala	Glu	Lys	Pro	Asp	Asp
91	His	Trp	Ala	Glu	Lys	Leu	Glu	Asp
92	His	Trp	Ala	Glu	Thr	Pro	Glu	Asp
93	Ser	Gln	Thr	Gln	Thr	Leu	Asp	Gly
94	Ser	Gln	Thr	Glu	Lys	Leu	Asp	Gly
95	Ser	Gln	Thr	Glu	Thr	Pro	Asp	Gly
96	Ser	Gln	Thr	Glu	Thr	Leu	Glu	Gly
97	Ser	Gln	Thr	Glu	Thr	Leu	Asp	Asp
98	Ser	Gln	Ala	Gln	Lys	Leu	Asp	Gly
99	Ser	Gln	Ala	Gln	Thr	Pro	Asp	Gly
100	Ser	Gln	Ala	Gln	Thr	Leu	Glu	Gly
101	Ser	Gln	Ala	Gln	Thr	Leu	Asp	Asp
102	Ser	Gln	Ala	Glu	Lys	Pro	Asp	Gly
103	Ser	Gln	Ala	Glu	Lys	Leu	Glu	Gly
104	Ser	Gln	Ala	Glu	Lys	Leu	Asp	Asp
105	Ser	Gln	Ala	Glu	Thr	Pro	Glu	Gly
106	Ser	Gln	Ala	Glu	Thr	Pro	Asp	Asp
107	Ser	Gln	Ala	Glu	Thr	Leu	Glu	Asp

108	Ser	Trp	Thr	Gln	Lys	Leu	Asp	Gly
109	Ser	Trp	Thr	Gln	Thr	Pro	Asp	Gly
110	Ser	Trp	Thr	Gln	Thr	Leu	Glu	Gly
111	Ser	Trp	Thr	Gln	Thr	Leu	Asp	Asp
112	Ser	Trp	Thr	Glu	Lys	Pro	Asp	Gly
113	Ser	Trp	Thr	Glu	Lys	Leu	Glu	Gly
114	Ser	Trp	Thr	Glu	Lys	Leu	Asp	Asp
115	Ser	Trp	Thr	Glu	Thr	Pro	Glu	Gly
116	Ser	Trp	Thr	Glu	Thr	Pro	Asp	Asp
117	Ser	Trp	Thr	Glu	Thr	Leu	Glu	Asp
118	Ser	Trp	Ala	Gln	Lys	Pro	Asp	Gly
119	Ser	Trp	Ala	Gln	Lys	Leu	Glu	Gly
120	Ser	Trp	Ala	Gln	Lys	Leu	Asp	Asp
121	Ser	Trp	Ala	Gln	Thr	Pro	Glu	Gly
122	Ser	Trp	Ala	Gln	Thr	Pro	Asp	Asp
123	Ser	Trp	Ala	Gln	Thr	Leu	Glu	Asp
124	Ser	Trp	Ala	Glu	Lys	Pro	Glu	Gly
125	Ser	Trp	Ala	Glu	Lys	Pro	Asp	Asp
126	Ser	Trp	Ala	Glu	Lys	Leu	Glu	Asp
127	Ser	Trp	Ala	Glu	Thr	Pro	Glu	Asp
128	His	Gln	Thr	Gln	Lys	Leu	Asp	Gly
129	His	Gln	Thr	Gln	Thr	Pro	Asp	Gly
130	His	Gln	Thr	Gln	Thr	Leu	Glu	Gly
131	His	Gln	Thr	Gln	Thr	Leu	Asp	Asp
132	His	Gln	Thr	Glu	Lys	Pro	Asp	Gly
133	His	Gln	Thr	Glu	Lys	Leu	Glu	Gly
134	His	Gln	Thr	Glu	Lys	Leu	Asp	Asp
135	His	Gln	Thr	Glu	Thr	Pro	Glu	Gly
136	His	Gln	Thr	Glu	Thr	Pro	Asp	Asp
137	His	Gln	Thr	Glu	Thr	Leu	Glu	Asp
138	His	Gln	Ala	Gln	Lys	Pro	Asp	Gly
139	His	Gln	Ala	Gln	Lys	Leu	Glu	Gly
140	His	Gln	Ala	Gln	Lys	Leu	Asp	Asp
141	His	Gln	Ala	Gln	Thr	Pro	Glu	Gly
142	His	Gln	Ala	Gln	Thr	Pro	Asp	Asp
143	His	Gln	Ala	Gln	Thr	Leu	Glu	Asp

144	His	Gln	Ala	Glu	Lys	Pro	Glu	Gly
145	His	Gln	Ala	Glu	Lys	Pro	Asp	Asp
146	His	Gln	Ala	Glu	Lys	Leu	Glu	Asp
147	His	Gln	Ala	Glu	Thr	Pro	Glu	Asp
148	His	Trp	Thr	Gln	Lys	Pro	Asp	Gly
149	His	Trp	Thr	Gln	Lys	Leu	Glu	Gly
150	His	Trp	Thr	Gln	Lys	Leu	Asp	Asp
151	His	Trp	Thr	Gln	Thr	Pro	Glu	Gly
152	His	Trp	Thr	Gln	Thr	Pro	Asp	Asp
153	His	Trp	Thr	Gln	Thr	Leu	Glu	Asp
154	His	Trp	Thr	Glu	Lys	Pro	Glu	Gly
155	His	Trp	Thr	Glu	Lys	Pro	Asp	Asp
156	His	Trp	Thr	Glu	Lys	Leu	Glu	Asp
157	His	Trp	Thr	Glu	Thr	Pro	Glu	Asp
158	His	Trp	Ala	Gln	Lys	Pro	Glu	Gly
159	His	Trp	Ala	Gln	Lys	Pro	Asp	Asp
160	His	Trp	Ala	Gln	Lys	Leu	Glu	Asp
161	His	Trp	Ala	Gln	Thr	Pro	Glu	Asp
162	His	Trp	Ala	Glu	Lys	Pro	Glu	Asp
163	His	Trp	Ala	Gln	Lys	Pro	Glu	Asp
164	His	Trp	Thr	Glu	Lys	Pro	Glu	Asp
165	His	Trp	Thr	Gln	Thr	Pro	Glu	Asp
166	His	Trp	Thr	Gln	Lys	Leu	Glu	Asp
167	His	Trp	Thr	Gln	Lys	Pro	Asp	Asp
168	His	Trp	Thr	Gln	Lys	Pro	Glu	Gly
169	His	Gln	Ala	Glu	Lys	Pro	Glu	Asp
170	His	Gln	Ala	Gln	Thr	Pro	Glu	Asp
171	His	Gln	Ala	Gln	Lys	Leu	Glu	Asp
172	His	Gln	Ala	Gln	Lys	Pro	Asp	Asp
173	His	Gln	Ala	Gln	Lys	Pro	Glu	Gly
174	His	Gln	Thr	Glu	Thr	Pro	Glu	Asp
175	His	Gln	Thr	Glu	Lys	Leu	Glu	Asp
176	His	Gln	Thr	Glu	Lys	Pro	Asp	Asp
177	His	Gln	Thr	Glu	Lys	Pro	Glu	Gly
178	His	Gln	Thr	Gln	Thr	Leu	Glu	Asp
179	His	Gln	Thr	Gln	Thr	Pro	Asp	Asp

180	His	Gln	Thr	Gln	Thr	Pro	Glu	Gly
181	His	Gln	Thr	Gln	Lys	Leu	Asp	Asp
182	His	Gln	Thr	Gln	Lys	Leu	Glu	Gly
183	His	Gln	Thr	Gln	Lys	Pro	Asp	Gly
184	Ser	Trp	Ala	Glu	Lys	Pro	Glu	Asp
185	Ser	Trp	Ala	Gln	Thr	Pro	Glu	Asp
186	Ser	Trp	Ala	Gln	Lys	Leu	Glu	Asp
187	Ser	Trp	Ala	Gln	Lys	Pro	Asp	Asp
188	Ser	Trp	Ala	Gln	Lys	Pro	Glu	Gly
189	Ser	Trp	Thr	Glu	Thr	Pro	Glu	Asp
190	Ser	Trp	Thr	Glu	Lys	Leu	Glu	Asp
191	Ser	Trp	Thr	Glu	Lys	Pro	Asp	Asp
192	Ser	Trp	Thr	Glu	Lys	Pro	Glu	Gly
193	Ser	Trp	Thr	Gln	Thr	Leu	Glu	Asp
194	Ser	Trp	Thr	Gln	Thr	Pro	Asp	Asp
195	Ser	Trp	Thr	Gln	Thr	Pro	Glu	Gly
196	Ser	Trp	Thr	Gln	Lys	Leu	Asp	Asp
197	Ser	Trp	Thr	Gln	Lys	Leu	Glu	Gly
198	Ser	Trp	Thr	Gln	Lys	Pro	Asp	Gly
199	Ser	Gln	Ala	Glu	Thr	Pro	Glu	Asp
200	Ser	Gln	Ala	Glu	Lys	Leu	Glu	Asp
201	Ser	Gln	Ala	Glu	Lys	Pro	Asp	Asp
202	Ser	Gln	Ala	Glu	Lys	Pro	Glu	Gly
203	Ser	Gln	Ala	Gln	Thr	Leu	Glu	Asp
204	Ser	Gln	Ala	Gln	Thr	Pro	Asp	Asp
205	Ser	Gln	Ala	Gln	Thr	Pro	Glu	Gly
206	Ser	Gln	Ala	Gln	Lys	Leu	Asp	Asp
207	Ser	Gln	Ala	Gln	Lys	Leu	Glu	Gly
208	Ser	Gln	Ala	Gln	Lys	Pro	Asp	Gly
209	Ser	Gln	Thr	Glu	Thr	Leu	Glu	Asp
210	Ser	Gln	Thr	Glu	Thr	Pro	Asp	Asp
211	Ser	Gln	Thr	Glu	Thr	Pro	Glu	Gly
212	Ser	Gln	Thr	Glu	Lys	Leu	Asp	Asp
213	Ser	Gln	Thr	Glu	Lys	Leu	Glu	Gly
214	Ser	Gln	Thr	Glu	Lys	Pro	Asp	Gly
215	Ser	Gln	Thr	Gln	Thr	Leu	Asp	Asp

216	Ser	Gln	Thr	Gln	Thr	Leu	Glu	Gly
217	Ser	Gln	Thr	Gln	Thr	Pro	Asp	Gly
218	Ser	Gln	Thr	Gln	Lys	Leu	Asp	Gly
219	His	Trp	Thr	Gln	Lys	Pro	Glu	Asp
220	His	Gln	Ala	Gln	Lys	Pro	Glu	Asp
221	His	Gln	Thr	Glu	Lys	Pro	Glu	Asp
222	His	Gln	Thr	Gln	Thr	Pro	Glu	Asp
223	His	Gln	Thr	Gln	Lys	Leu	Glu	Asp
224	His	Gln	Thr	Gln	Lys	Pro	Asp	Asp
225	His	Gln	Thr	Gln	Lys	Pro	Glu	Gly
226	Ser	Trp	Ala	Gln	Lys	Pro	Glu	Asp
227	Ser	Trp	Thr	Glu	Lys	Pro	Glu	Asp
228	Ser	Trp	Thr	Gln	Thr	Pro	Glu	Asp
229	Ser	Trp	Thr	Gln	Lys	Leu	Glu	Asp
230	Ser	Trp	Thr	Gln	Lys	Pro	Asp	Asp
231	Ser	Trp	Thr	Gln	Lys	Pro	Glu	Gly
232	Ser	Gln	Ala	Glu	Lys	Pro	Glu	Asp
233	Ser	Gln	Ala	Gln	Thr	Pro	Glu	Asp
234	Ser	Gln	Ala	Gln	Lys	Leu	Glu	Asp
235	Ser	Gln	Ala	Gln	Lys	Pro	Asp	Asp
236	Ser	Gln	Ala	Gln	Lys	Pro	Glu	Gly
237	Ser	Gln	Thr	Glu	Thr	Pro	Glu	Asp
238	Ser	Gln	Thr	Glu	Lys	Leu	Glu	Asp
239	Ser	Gln	Thr	Glu	Lys	Pro	Asp	Asp
240	Ser	Gln	Thr	Glu	Lys	Pro	Glu	Gly
241	Ser	Gln	Thr	Gln	Thr	Leu	Glu	Asp
242	Ser	Gln	Thr	Gln	Thr	Pro	Asp	Asp
243	Ser	Gln	Thr	Gln	Thr	Pro	Glu	Gly
244	Ser	Gln	Thr	Gln	Lys	Leu	Asp	Asp
245	Ser	Gln	Thr	Gln	Lys	Leu	Glu	Gly
246	Ser	Gln	Thr	Gln	Lys	Pro	Asp	Gly
247	His	Gln	Thr	Gln	Lys	Pro	Glu	Asp
248	Ser	Trp	Thr	Gln	Lys	Pro	Glu	Asp
249	Ser	Gln	Ala	Gln	Lys	Pro	Glu	Asp
250	Ser	Gln	Thr	Glu	Lys	Pro	Glu	Asp
251	Ser	Gln	Thr	Gln	Thr	Pro	Glu	Asp

252	Ser	Gln	Thr	Gln	Lys	Leu	Glu	Asp
253	Ser	Gln	Thr	Gln	Lys	Pro	Asp	Asp
254	Ser	Gln	Thr	Gln	Lys	Pro	Glu	Gly
255	Ser	Gln	Thr	Gln	Lys	Pro	Glu	Asp

Other preferred proteins are those wherein Xaa at position 27 is Ala; Xaa at position 77 is Ser; Xaa at position 118 is Gly; and the amino residues at positions 97, 100, 101, 105, 106, 107, 108 and 111 are as described in Table I.

The present invention provides biologically active proteins that provide effective treatment for obese type II diabetics. Unexpectedly, the leptin proteins of Table I have improved properties due to specific substitutions to the human obesity protein. These proteins are more stable than both the mouse and human obesity protein and, therefore, represent superior therapeutic agents.

Experiments were performed with five to six month old male, inbred normal ICR mice, inbred normal(*ob/+*), obese-diabetic mice (*ob/ob*) from the Jackson Laboratories (Bar Harbor, Maine) or Harlan (England), and obese-diabetic (*db/db*) mice.

Both normal and diabetic mice were housed three or six per plastic cage (with bedding) and water and feed were available *ad libitum*. The temperature of animal rooms was maintained at 23 ± 2°C and lights were on from 0600 to 1800 h. Blood samples were collected from the tail vein. The most closely related biological test is, therefore, to inject the test article by any of several routes of administration (e.g., i.v., s.c., i.p., or by minipump or cannula) and then to monitor food and water consumption, body weight gain, plasma chemistry or hormones (glucose, insulin, ACTH, corticosterone, GH, T4) over various time periods. Suitable test animals include normal mice (ICR, etc.) and obese mice (*ob/ob*, *Avy/a*, KK-Ay, tubby, fat). Controls for nonspecific effects for these injections can be done using vehicle with or without test articles of similar composition in the same

animal monitoring the same parameters or the test article itself in animals that are thought to lack the receptor (db/db mice, fa/fa or cp/cp rats).

Blood glucose levels were measured by a glucose oxidase method or a coupled hexokinase method. Plasma insulin was determined with radioimmunoassay kits using rat insulin as the standard. Plasma triglycerides were measured using commercial kits with glycerol as the standard.

The foregoing studies demonstrated that leptin and leptin mimetis, regulated food intake and body weight in normal ICR and genetically obese *ob/ob* mice. Chronic administration of leptin and leptin mimetics to *ob/ob* mice totally ameliorated the diabetic state of these animals showing the potential promise for these anti-diabetic proteins as a treatment for obese type II diabetics. Thus, the present invention provides methods for treating obese type II diabetics, particularly those with low circulating levels of leptin.

The compounds of the present invention may be produced by well known chemical procedures, such as solution or solid-phase peptide synthesis, or semi-synthesis in solution beginning with protein fragments coupled through conventional solution methods. Such methods are well known in the art and may be found in general texts in the area such as H. Dugas and C. Penney, BIOORGANIC CHEMISTRY, (1981) at pages 54-92.

Proteins useful in the presently claimed methods also may be prepared by well known recombinant DNA techniques such as those described in Maniatis, et al. (1988) Molecular Cloning: A Laboratory Manual, Cold Spring Harbor Press, Cold Spring Harbor Laboratory, Cold Spring Harbor, New York or Current Protocols in Molecular Biology (1989) and supplements. Techniques for making substitutional mutations at predetermined sites in DNA having a known sequence are well known, for example M13 primer mutagenesis. The mutations that might be made in the DNA encoding the present anti-diabetic proteins must not place the sequence out of

reading frame and preferably will not create complementary regions that could produce secondary mRNA structure. See, DeBoer, et al., European Patent Publication, 075,444 A (1983).

The present invention provides a method for treating obese type II diabetics. The method comprises administering an effective amount of an leptin or leptin mimetic in a dose between about 1 and 10,000 μg/kg. A preferred dose is from about 20 to 10,000 μg/kg. A more preferred dose is from about 200 to 600 μg/kg. In practicing this method, anti-diabetic proteins can be administered in a single daily dose or in multiple doses per day. The treatment regime may require administration over extended periods of time. The amount per administered dose or the total amount administered will be determined by the physician and depend on such factors as the mass of the patient, the age and general health of the patient and the tolerance of the patient to the compound.

The instant invention further provides pharmaceutical formulations comprising compounds of the present invention. The proteins, preferably in the form of a pharmaceutically acceptable salt, can be formulated for parenteral administration. For example, compounds can be admixed with conventional pharmaceutical carriers and excipients. The compositions comprising claimed proteins contain from about 0.1 to 90% by weight of the active protein, preferably in a soluble form, and more generally from about 10 to 30%. Furthermore, the present proteins may be administered alone or in combination with other anti-obesity agents or agents useful in treating diabetes.

For intravenous use, the protein is administered in commonly used intravenous fluids and administered by infusion. For intramuscular preparations, a sterile formulation, preferably a suitable soluble salt form of the protein, for example the hydrochloride salt, can be dissolved and administered in a pharmaceutical diluent such as pyrogen-free water or physiological saline. A suitable insoluble

form of the compound may be prepared and administered as a suspension in an aqueous base or a pharmaceutically acceptable oil base, e.g. an ester of a long chain fatty acid such as ethyl oleate.

In a preferred embodiment, the present invention provides a method for treating obese type II diabetics with low leptin levels, though diabetic patients with high endogenous leptin levels may also benefit from the presently claimed methods. Methods for assaying serum and plasma leptin levels may be accomplished using standard antibody-based methodologies. Leptin assay kits are also commercially available from Linco Research, Inc. (14 Research Park Dr., St Louis, MO 63304)

Treating obese type II diabetics having leptin levels between 0 and 80 ng/ml is preferred. More preferred is to treat obese type II diabetics having leptin levels between 0 and 50 ng/ml. More highly preferred is to treat obese type II diabetics having leptin levels between 0 and 30 ng/ml. Most preferred is to treat obese type II diabetics having leptin levels between 0 and 15 ng/ml.

By way of illustration, the following examples are provided to help describe how to make and practice the various embodiments of the invention. These example are in no way meant to limit the scope of the invention.

Example 1

A DNA sequence encoding the following protein sequence:

Met-Arg-SEQ ID NO:4.

was obtained using standard PCR methodology from a human fat cell library (commercially available from CLONETECH). Briefly, degenerate primers were designed based on the published amino acid sequence of the human *ob* gene. The primers were prepared for use in polymerase chain reaction (PCR) amplification methods using a Model 380A DNA synthesizers (PE-Applied Biosystems, Inc., 850 Lincoln Center

Drive, Foster City, CA 94404). Forward primers OB.F1M (5-GG GG CAT ATG AGG GTA CCT ATC CAG AAA GTC CAG GAT GAC AC)and OB.F2H (5-GG GG CAT ATG AGG GTA CCC ATC CAG AAG GTG CAG GAC GA)(and reverse primers OB.R1M (5-GG GG GGATC GAT AAT TTA GCA TCC AGG GCT AAG ATC CAA CTG CCA AAG CAT) and OB.R2H (5-GG GG GGATC CTA TTA GCA CCC GGG AGA CAG GTC CAG CTG CCA CAA CAT) were mixed together with a PCR-ready human fat cell cDNA as the template (Clontech Laboratories, Inc., 4030 Fabian Way, Palo Alto, CA 94303; Item #7128-1).

The 2 sets of PCR amplifications were performed using 2.5 units of Amplitag DNA polymerase (Perkin Elmer Cetus) or 2 units of Vent DNA polymerase (New England Biolabs) in 100 uL reactions. PCR reactions contained 1 uL of human fat cell cDNA, 10 pmol of each primer (all four were mixed). The following conditions were used for "Touchdown PCR": 2 cycles: 94°Cx30 sec, 60°Cx30 sec, 72°Cx45 sec 2 cycles: 94°Cx30 sec, 56°Cx30 sec, 72°Cx45 sec; 2cycles: 94°Cx30 sec, 52°Cx30 sec, 72°Cx45 sec; 2cycles: 94°Cx30 sec, 48°Cx30 sec, 72°Cx45 sec; 2 cycles: 94°Cx30 sec, 44°Cx30 sec, 72°Cx45 sec: 28 cycles 94°Cx30 sec, 52°Cx30 sec, 72°Cx45 sec

The resultant PCR reactions products were run on a 1% agarose gel and a band of an approximate 450 bp in size was visualized by ethidium bromide staining. This band was present in both sets of PCR reactions. The bands were excised and reamplified using above conditions in 30 cycles (94x30 sec, 52x30, 72x45). The PCR product obtained using Vent DNA polymerase was gel purified and cloned into a pCR-SCRIPT cloning vector (Stratagene). The vector was then used to transform E. coli cells. Plasmid DNA was isolated from 20 white colonies of E. coli and samples from three clones were sequenced. Two such colonies, E. coli DH10B/pOJ717 and E. coli DH10B/pOJ718 were deposited with the Northern Regional Research Laboratories (NRRL) under terms of the Budapest Treaty and are available under Accession Numbers B-21408 and B-21409 respectively.

Example 2

Vector Construction

A plasmid containing the DNA sequence encoding a desired protein is constructed to include NdeI and BamHI restriction sites. The plasmid carrying the cloned PCR product is digested with NdeI and BamHI restriction enzymes. The small ~ 450bp fragment is gel-purified and ligated into the vector pRB182 from which the coding sequence for A-C-B proinsulin is deleted. The ligation products are transformed into E. coli DH10B (commercially available) and colonies growing on tryptone-yeast plates supplemented with 10 µg/mL of tetracycline are analyzed. Plasmid DNA is isolated, digested with NdeI and BamHI and the resulting fragments are separated by agarose gel electrophoresis. Plasmids containing the expected ~ 450bp NdeI to BamHI fragment are kept. E. coli B BL21 (DE3) are transformed with this second plasmid expression suitable for culture for protein production.

The techniques of transforming cells with the aforementioned vectors are well known in the art and may be found in such general references as Maniatis, et al., MOLECULAR CLONING: A LABORATORY MANUAL, Cold Spring Harbor Press, Cold Spring Harbor Laboratory, Cold Spring Harbor, New York (1988), or CURRENT PROTOCOLS IN MOLECULAR BIOLOGY, (F. Ausabel, ed., 1989) and supplements thereof. The techniques involved in the transformation of E. coli cells used in the preferred practice of the invention as exemplified herein are well known in the art. The precise conditions under which the transformed E. coli cells are cultured is dependent on the nature of the E. coli host cell line and the expression or cloning vectors employed. For example, vectors which incorporate thermoinducible promoter-operator regions, such as the c1857 thermoinducible lambda-phage promoter-operator region, require a temperature shift from about 30°C to about 40°C in the culture conditions so as to induce protein synthesis.

In the preferred embodiment of the invention E. coli K12 RV308 cells are employed as host cells but numerous other cell lines are available such as, but not limited to, E. coli K12 L201, L687, L693, L507, L640, L641, L695, L814 (E. coli B). The transformed host cells are then plated on appropriate media under the selective pressure of the antibiotic corresponding to the resistance gene present on the expression plasmid. The cultures are then incubated for a time and temperature appropriate to the host cell line employed.

Proteins which are expressed in high-level bacterial expression systems characteristically aggregate in granules or inclusion bodies which contain high levels of the overexpressed protein. See, e.g., Kreuger et al., PROTEIN FOLDING, (Gierasch and King, eds., 1990) at pages 136-142, American Association for the Advancement of Science Publication No. 89-18S, Washington, D.C. Such protein aggregates must be solubilized to provide further purification and isolation of the desired protein product. Id. A variety of techniques using strongly denaturing solutions such as guanidinium-HCl and/or weakly denaturing solutions such as dithiothreitol (DTT) are used to solubilize the proteins.

Gradual removal of the denaturing agents (often by dialysis) in a solution allows the denatured protein to assume its native conformation. The particular conditions for denaturation and folding are determined by the particular protein expression system and/or the protein in question.

Preferably, the present proteins are expressed as Met-Arg-SEQ ID NO: X so that the expressed proteins may be readily converted to the claimed protein with cathepsin C (also known as diaminopeptidase). The purification of proteins is by techniques known in the art and includes reverse phase chromatography, affinity chromatography, and size exclusion chromatography.

The claimed proteins contain two cysteine residues. Thus, a di-sulfide bond may be formed to stabilize the

protein. The present invention includes proteins wherein the Cys at position 96 is crosslinked to Cys at position 146 as well as those proteins without such di-sulfide bonds.

In addition the proteins useful in the present invention may exist, particularly when formulated, as dimers, trimers, tetramers, and other multimers. Such multimers are included within the scope of the present invention.

I Claim:

1. Use of a leptin or leptin mimetic for the manufacture of a medicament for treating or preventing diabetes mellitus.

2. A method of **Claim 1** wherein said leptin or leptin mimetic is selected from the group consisting of

(a)

```
Val Pro Ile Gln Lys Val Gln Asp Asp Thr Lys Thr Leu Ile Lys Thr
1               5                  10                  15

Ile Val Thr Arg Ile Asn Asp Ile Ser His Thr Xaa Ser Val Ser Ser
            20                  25                  30

Lys Gln Lys Val Thr Gly Leu Asp Phe Ile Pro Gly Leu His Pro Ile
        35                  40                  45

Leu Thr Leu Ser Lys Met Asp Gln Thr Leu Ala Val Tyr Gln Gln Ile
    50                  55                  60

Leu Thr Ser Met Pro Ser Arg Asn Val Ile Gln Ile Ser Asn Asp Leu
65                  70                  75                  80

Glu Asn Leu Arg Asp Leu Leu His Val Leu Ala Phe Ser Lys Ser Cys
                85                  90                  95

His Leu Pro Qln Ala Ser Gly Leu Glu Thr Leu Glu Ser Leu Gly Gly
            100                 105                 110

Val Leu Glu Ala Ser Gly Tyr Ser Thr Glu Val Val Ala Leu Ser Arg
        115                 120                 125

Leu Gln Gly Ser Leu Gln Asp Met Leu Gln Gln Leu Asp Leu Ser Pro
    130                 135                 140

Gly Cys
145
```

wherein:

Xaa at position 28 is Gln or absent;

(SEQ ID NO:1)

(b)

```
Val Pro Ile Trp Arg Val Gln Asp Asp Thr Lys Thr Leu Ile Lys Thr
  1               5                  10                  15

Ile Val Thr Arg Ile Ser Asp Ile Ser His Met Gln Ser Val Ser Ser
            20                  25                  30

Lys Gln Arg Val Thr Gly Leu Asp Phe Ile Pro Gly Leu His Pro Val
```

35 40 45

Leu Ser Leu Ser Lys Met Asp Gln Thr Leu Ala Ile Tyr Gln Gln Ile
50 55 60

Leu Thr Ser Leu Pro Ser Arg Asn Val Ile Gln Ile Ser Asn Asp Leu
65 70 75 80

Glu Asn Leu Arg Asp Leu Leu His Leu Leu Ala Ser Ser Lys Ser Cys
85 90 95

Pro Leu Pro Gln Ala Arg Ala Leu Glu Thr Leu Glu Ser Leu Gly Gly
100 105 110

Val Leu Glu Ala Ser Leu Tyr Ser Thr Glu Val Val Ala Leu Ser Arg
115 120 125

Leu Gln Gly Ala Leu Gln Asp Met Leu Arg Gln Leu Asp Leu Ser Pro
130 135 140

Gly Cys
145 ; (SEQ ID NO:2)

(c)

Val Pro Ile Cys Lys Val Gln Asp Asp Thr Lys Thr Leu Ile Lys Thr
1 5 10 15

Ile Val Thr Arg Ile Asn Asp Ile Ser His Thr Xaa Ser Val Ser Ser
20 25 30

Lys Gln Arg Val Thr Gly Leu Asp Phe Ile Pro Gly Leu His Pro Leu
35 40 45

Leu Ser Leu Ser Lys Met Asp Gln Thr Leu Ala Ile Tyr Gln Gln Ile
50 55 60

Leu Thr Ser Leu Pro Ser Arg Asn Val Val Gln Ile Ser Asn Asp Leu
65 70 75 80

Glu Asn Leu Arg Asp Leu Leu His Leu Leu Ala Ala Ser Lys Ser Cys
85 90 95

Pro Leu Pro Gln Val Arg Ala Leu Glu Ser Leu Glu Ser Leu Gly Val
100 105 110

Val Leu Glu Ala Ser Leu Tyr Ser Thr Glu Val Val Ala Leu Ser Arg
115 120 125

Leu Gln Gly Ser Leu Gln Asp Met Leu Arg Gln Leu Asp Leu Ser Pro
130 135 140

Gly Cys
145

wherein Xaa at position 28 is Gln or absent; (SEQ ID NO:3)

(d)

```
Val Pro Ile Gln Lys Val Gln Asp Asp Thr Lys Thr Leu Ile Lys Thr
1               5                  10                  15

Ile Val Thr Arg Ile Asn Asp Ile Ser His Xaa Xaa Ser Val Ser Ser
            20                  25                  30

Lys Gln Lys Val Thr Gly Leu Asp Phe Ile Pro Gly Leu His Pro Ile
        35                  40                  45

Leu Thr Leu Ser Lys Met Asp Gln Thr Leu Ala Val Tyr Gln Gln Ile
    50                  55                  60

Leu Thr Ser Met Pro Ser Arg Asn Val Ile Gln Ile Ser Asn Asp Leu
65                  70                  75                  80

Glu Asn Leu Arg Asp Leu Leu His Val Leu Ala Phe Ser Lys Ser Cys
                85                  90                  95

His Leu Pro Trp Ala Ser Gly Leu Glu Thr Leu Asp Ser Leu Gly Gly
            100                 105                 110

Val Leu Glu Ala Ser Gly Tyr Ser Thr Glu Val Val Ala Leu Ser Arg
        115                 120                 125

Leu Gln Gly Ser Leu Gln Asp Met Leu Trp Gln Leu Asp Leu Ser Pro
    130                 135                 140

145
Gly Cys
```

wherein:

Xaa at position 27 is Thr or Ala; and

Xaa at position 28 is Gln or absent;

(SEQ ID NO:4)

(e)

```
Val Pro Ile Gln Lys Val Gln Ser Asp Thr Lys Thr Leu Ile Lys
1               5                  10                  15

Thr Ile Val Thr Arg Ile Asn Asp Ile Ser His Thr Gln Ser Val
            20                  25                  30

Ser Ser Lys Gln Arg Val Thr Gly Leu Asp Phe Ile Pro Gly Leu
        35                  40                  45

His Pro Val Leu Thr Leu Ser Gln Met Asp Gln Thr Leu Ala Ile
    50                  55                  60

Tyr Gln Gln Ile Leu Ile Asn Leu Pro Ser Arg Asn Val Ile Gln
65                  70                  75

Ile Ser Asn Asp Leu Glu Asn Leu Arg Asp Leu Leu His Leu Leu
                80                  85                  90
```

Ala Phe Ser Lys Ser Cys His Leu Pro Leu Ala Ser Gly Leu Glu
95 100 105

Thr Leu Glu Ser Leu Gly Asp Val Leu Glu Ala Ser Leu Tyr Ser
110 115 120

Thr Glu Val Val Ala Leu Ser Arg Leu Gln Gly Ser Leu Gln Asp
125 130 135

Met Leu Trp Gln Leu Asp Leu Ser Pro Gly Cys
140 145 ; (SEQ ID NO:5)

(f)

Val Pro Ile His Lys Val Gln Asp Asp Thr Lys Thr Leu Ile Lys Thr
1 5 10 15

Ile Val Thr Arg Ile Asn Asp Ile Ser His Thr Gln Ser Val Ser Ala
20 25 30

Arg Gln Arg Val Thr Gly Leu Asp Phe Ile Pro Gly Leu His Pro Ile
35 40 45

Leu Ser Leu Ser Lys Met Asp Gln Thr Leu Ala Val Tyr Gln Gln Ile
50 55 60

Leu Thr Ser Leu Pro Ser Gln Asn Val Leu Gln Ile Ala His Asp Leu
65 70 75 80

Glu Asn Leu Arg Asp Leu Leu His Leu Leu Ala Phe Ser Lys Ser Cys
85 90 95

Ser Leu Pro Gln Thr Arg Gly Leu Gln Lys Pro Glu Ser Leu Asp Gly
100 105 110

Val Leu Glu Ala Ser Leu Tyr Ser Thr Glu Val Val Ala Leu Ser Arg
115 120 125

Leu Gln Gly Ser Leu Gln Asp Ile Leu Gln Gln Leu Asp Leu Ser Pro
130 135 140

Glu Cys

145, (SEQ ID NO:5)

(g)

Val Pro Ile Gln Lys Val Gln Asp Asp Thr Lys Thr Leu Ile Lys Thr
1 5 10 15

Ile Val Thr Arg Ile Xaa Asp Ile Ser His Xaa Xaa Ser Val Ser Ser
20 25 30

Lys Gln Lys Val Thr Gly Leu Asp Phe Ile Pro Gly Leu His Pro Ile
35 40 45

Leu Thr Leu Ser Lys Xaa Asp Gln Thr Leu Ala Val Tyr Gln Gln Ile
50 55 60

Leu Thr Ser Xaa Pro Ser Arg Xaa Val Ile Gln Ile Xaa Asn Asp Leu
65 70 75 80

Glu Asn Leu Arg Asp Leu Leu His Val Leu Ala Phe Ser Lys Ser Cys
85 90 95

His Leu Pro Trp Ala Ser Gly Leu Glu Thr Leu Asp Ser Leu Gly Gly
100 105 110

Val Leu Glu Ala Ser Xaa Tyr Ser Thr Glu Val Val Ala Leu Ser Arg
115 120 125

Leu Gln Gly Ser Leu Gln Asp Met Leu Trp Gln Leu Asp Leu Ser Pro
130 135 140

145
Gly Cys

wherein:

Xaa at position 22 is Asn, Asp or Glu;

Xaa at position 27 is Thr or Ala;

Xaa at position 28 is Gln, Glu, or absent;

Xaa at position 54 is Met or Ala;

Xaa at position 68 is Met or Leu;

Xaa at position 72 Asn, Asp or Glu;

Xaa at position 77 is Ser or Ala;

Xaa at position 118 is Gly or Leu;

said protein having at least one substitution selected from the group consisting of:

His at position 97 is replaced with Ser or Pro;

Trp at position 100 is replaced with Gln, Ala or Leu;

Ala at position 101 is replaced with Thr or Val;

Ser at position 102 is replaced with Arg;

Gly at position 103 is replaced with Ala;

Glu at position 105 is replaced with Gln;

Thr at position 106 is replaced with Lys or Ser;

Leu at position 107 is replaced with Pro;

Asp at position 108 is replaced with Glu; or

Gly at position 111 is replaced with Asp; and,

(SEQ ID NO:7)

(h)

Val Pro Ile Gln Lys Val Gln Asp Asp Thr Lys Thr Leu Ile Lys Thr

1 5 10 15

Ile Val Thr Arg Ile Asn Asp Ile Ser His Xaa Gln Ser Val Ser Ser
20 25 30

Lys Gln Lys Val Thr Gly Leu Asp Phe Ile Pro Gly Leu His Pro Ile
35 40 45

Leu Thr Leu Ser Lys Met Asp Gln Thr Leu Ala Val Tyr Gln Gln Ile
50 55 60

Leu Thr Ser Met Pro Ser Arg Asn Val Ile Gln Ile Xaa Asn Asp Leu
65 70 75 80

Glu Asn Leu Arg Asp Leu Leu His Val Leu Ala Phe Ser Lys Ser Cys
85 90 95

His Leu Pro Trp Ala Ser Gly Leu Glu Thr Leu Asp Ser Leu Gly Gly
100 105 110

Val Leu Glu Ala Ser Xaa Tyr Ser Thr Glu Val Val Ala Leu Ser Arg
115 120 125

Leu Gln Gly Ser Leu Gln Asp Met Leu Trp Gln Leu Asp Leu Ser Pro
130 135 140

145
Gly Cys

wherein:

Xaa at position 27 is Thr or Ala;

Xaa at position 77 is Ser or Ala;

Xaa at position 118 is Gly or Leu;

said protein having at least one substitution, preferably having one to five substitutions and, most preferably, one to two substitutions selected from the group consisting of:

His at position 97 is replaced with Ser;

Trp at position 100 is replaced with Gln;

Ala at position 101 is replaced with Thr;

Glu at position 105 is replaced with Gln;

Thr at position 106 is replaced with Lys;

Leu at position 107 is replaced with Pro;

Asp at position 108 is replaced with Glu; or

Gly at position 111 is replaced with Asp,

or a pharmaceutically acceptable salt or solvate thereof.

(SEQ ID NO:8)

3. A method as claimed in any one of **Claims 1 or 2** wherein the diabetes mellitus is associated with high endogenous leptin levels.

4. A method as claimed in any one of **Claims 1 or 3** wherein the diabetes mellitus is associated with low endogenous leptin levels.

5. A method as claimed in any one of **Claims 1 or 3** wherein said obese type II diabetic has endogenous leptin levels in the range of 0 to 80 ng/ml.

6. A method as claimed in any one of **Claims 1 or 3** wherein said obese type II diabetic has endogenous leptin levels in the range of 0 to 50 ng/ml.

7. A method as claimed in any one of **Claims 1 or 3** wherein said obese type II diabetic has endogenous leptin levels in the range of 0 to 30 ng/ml.

8. A method as claimed in any one of **Claims 1 or 3** wherein said obese type II diabetic has endogenous leptin levels in the range of 0 to 15 ng/ml.

9. A method for treating or preventing diabetes mellitus which comprises administering to an afflicted patient a leptin or leptin mimetic.

10. The method of **Claim 9** wherein said leptin or leptin mimetic is selected from the group consisting of: SEQ ID NO:1, SEQ ID NO:2, SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:5, SEQ ID NO:6, SEQ ID NO:7, and SEQ ID NO:8.

11. The method as claimed in any one of **Claims 9 or 10** wherein the diabetes mellitus is associated with high endogenous leptin levels.

12. The method as claimed in any one of **Claims 9 or 10** wherein the diabetes mellitus is associated with low endogenous leptin levels.

13. The method as claimed in any one of **Claims 9 or 10** wherein said obese type II diabetic has endogenous leptin levels in the range of 0 to 80 ng/ml.

14. The method as claimed in any one of **Claims 9 or 10** wherein said obese type II diabetic has endogenous leptin levels in the range of 0 to 50 ng/ml.

15. The method as claimed in any one of **Claims 9 or 10** wherein said obese type II diabetic has endogenous leptin levels in the range of 0 to 30 ng/ml.

16. The method as claimed in any one of **Claims 9 or 10** wherein said obese type II diabetic has endogenous leptin levels in the range of 0 to 15 ng/ml.

17. A formulation for treating or preventing diabetes mellitus, comprising as an active ingredient a leptin or leptin mimetic.

18. A formulation of **Claim 17** wherein said leptin or leptin mimetic is selected from the group consisting of: SEQ ID NO:1, SEQ ID NO:2, SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:5, SEQ ID NO:6, SEQ ID NO:7, and SEQ ID NO:8.

19. The method as claimed in any one of **Claims 17 or 18** wherein the diabetes mellitus is associated with low or high endogenous leptin levels.