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(54) PREVENTIVE/REMEDY FOR ARTERIOSCLEROSIS

(57) A scavenger receptor A expression down-regulator and a drug for preventing or treating arteriosclerosis which contain, as the active ingredient, a C-terminal globular domain of adiponectin, adiponectin, or a gene encoding the domain or adiponectin.

According to the present invention, there is provided a preventive or therapeutic agent capable of directly preventing intimal thickening, which constitutes an essential feature of arteriosclerosis. This effect can be attained through arresting the onset and development of arteriosclerosis by reducing the expression level of scavenger receptor A in arterial walls and preventing lipid buildup in macrophages.

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Description

Technical Field

[0001] The present invention relates to a drug for preventing or treating arteriosclerosis.

Background Art

[0002] The term "arteriosclerosis" refers to a pathological condition where the walls of an artery lose elasticity and become brittle. Arteriosclerosis is one of key factors causing aduet diseases, including cerebral hemorrhage, cerebral infarction, myocardial infarction, and nephrosclerosis. Known causes of arteriosclerosis include hyperlipidemia, and bacteria, viruses, or lipid peroxide in blood. However, the pathogenesis of arteriosclerosis has not yet been fully elucidated. In any case, since arteriosclerosis has been observed to begin with thickening of arterial walls caused by damage to the arterial intima or endothelium, there is keen demand for development of a drug capable of inhibiting thickening of the arterial intima.

[0003] Accordingly, an object of the present invention is to provide a drug which is effective for preventing or treating arteriosclerosis.

Disclosure of the Invention

[0004] Under the above circumstance, the present inventors have devoted their research efforts to pharmacological actions of adiponectin, which is known to have an insulin resistance reducing effect, and have found that adiponectin-gene-deficient mice show significantly thickened arterial intima; and that adiponectin, a C-terminal globular domain thereof, or a gene thereof is useful as a preventive or therapeutic drug for arteriosclerosis, on the basis of their experimental results that when apoE-deficient mice, which are employed as atherosclerosis onset model mice, are manipulated to over-express adiponectin, in particular, the C-terminal globular domain of adiponectin, the onset of arteriosclerosis is suppressed. In addition, since over-expression of adiponectin lowers the expression level of scavenger receptor A without significantly affecting the levels of free fatty acid, neutral fat, or total cholesterol in blood, the inventors have concluded that the arteriosclerosis preventive action of adiponectin lowers the expression level of scavenger receptor A, whereby accumulation of lipids in macrophages is prevented. The present invention has been accomplished on the basis of these findings.

[0005] Accordingly, the present invention provides a drug for preventing or treating arteriosclerosis, the drug containing, as an active ingredient thereof, a C-terminal globular domain of adiponectin, adiponectin, or a gene encoding the C-terminal globular domain of adiponectin or the adiponectin.

[0006] The present invention also provides a scavenger receptor A expression down-regulator which contains, as an active ingredient thereof, a C-terminal globular domain of adiponectin, adiponectin, or a gene encoding the C-terminal globular domain of adiponectin or the adiponectin.

[0007] The present invention also provides use, in the manufacture of a drug for preventing or treating arteriosclerosis, of a C-terminal globular domain of adiponectin, adiponectin, or a gene encoding the C-terminal globular domain of adiponectin or the adiponectin.

[0008] The present invention also provides use, in the manufacture of a scavenger receptor A expression down-regulator, of a C-terminal globular domain of adiponectin, adiponectin, or a gene encoding the C-terminal globular domain of adiponectin or the adiponectin.

[0009] The present invention also provides a method for treating arteriosclerosis, comprising administering, to a subject in need thereof, an effective amount of a C-terminal globular domain of adiponectin, adiponectin, or a gene encoding the C-terminal globular domain of adiponectin or the adiponectin.

[0010] The present invention also provides a method for down-regulating the expression level of scavenger receptor A in a patient, comprising administering to the patient an effective amount of a C-terminal globular domain of adiponectin, adiponectin, or a gene encoding the C-terminal globular domain of adiponectin or the adiponectin.

Brief Description of the Drawings

[0011]

Fig. 1 schematically shows a gene targeting performed on adiponectin gene deficiency, in which, a restriction map of a mouse adiponectin gene (top), an adiponectin gene targeting vector (middle), and a deduced targeting allele (bottom).

Fig. 2 shows the results of Southern blotting of ES-cell-derived DNA samples which have been digested with SpeI

and EoRV. The bands of 17 kb are obtained from wild-type alleles, and those of 10.5 kb are from mutated alleles.
Fig. 3 shows the results of Southern blotting of SpeI- and EoRV-digested DNA samples from a wild-type mouse, a hetero-deficient (adipo +/-) mouse, and a homo-deficient (adipo -/-) mouse. The bands of 17 kb are obtained from wild-type alleles, and those of 10.5 kb are from mutated alleles. Fig. 4 shows the results of Northern blotting of white adipose tissue samples from a wild-type mouse, a hetero-deficient (adipo +/-) mouse, and a homo-deficient (adipo -/-) mouse.
Fig. 5 shows blood adiponectin level of a wild-type mouse, a hetero-deficient (adipo +/-) mouse, and a homo-deficient (adipo -/-) mouse. **P<0.01.
Fig. 6 shows blood leptin level of a wild-type mouse, a hetero-deficient (adipo +/-) mouse, and a homo-deficient (adipo -/-) mouse.
Fig. 7 shows the body weight, at 6 weeks of age, of a wild-type mouse, a hetero-deficient (adipo +/-) mouse, and a homo-deficient (adipo -/-) mouse.
Fig. 8 shows the results of an insulin tolerance test performed on a wild-type mouse and a hetero-deficient (adipo +/-) mouse at 6 weeks of age. *P<0.05.
Fig. 9 shows the results of a glucose tolerance test performed on a wild-type mouse and a hetero-deficient (adipo +/-) mouse at 6 weeks of age. *P<0.05.
Fig. 10 shows the results of a glucose tolerance test performed on a wild-type mouse and a hetero-deficient (adipo +/-) mouse after having loaded with a high-fat diet for 10 weeks. *P<0.05, **P<0.01.
Fig. 11 shows the results of an insulin tolerance test performed on a wild-type mouse and a homo-deficient (adipo - /-) mouse, at 6 weeks of age. *P<0.05, **P<0.01.
Fig. 12 shows the results of a glucose tolerance test performed on a wild-type mouse and a hetero-deficient (adipo +/-) mouse, at 6 weeks of age. *P<0.05, **P<0.01.
Fig. 13 shows levels, in blood, of free fatty acid (FFA), neutral fat (TG), total cholesterol (TC) of a wild-type mouse and a hetero-deficient (adipo +/-) mouse.
Fig. 14 shows levels, in blood, of free fatty acid (FFA), neutral fat (TG), total cholesterol (TC) of a wild-type mouse and a homo-deficient (adipo -/-) mouse.
Fig. 15 shows the inner diameter of a blood vessel of a wild-type mouse and a hetero-deficient (adipo +/-) mouse, as measured two weeks after the mice underwent cuff placement.
Fig. 16 shows the degree of intimal thickening of a wild-type mouse and a hetero-deficient (adipo +/-) mouse, as measured two weeks after the mice underwent cuff placement.
Fig. 17 shows the degree of medial thickening of a wild-type mouse and a hetero-deficient (adipo +/-) mouse, as measured two weeks after the mice underwent cuff placement.
Fig. 18 shows the intima/media ratio of a wild-type mouse and a hetero-deficient (adipo +/-) mouse, as measured two weeks after the mice underwent cuff placement.
Fig. 19 shows the foci of arteriosclerosis in an apoE-deficient (apoE-/-:a) mouse and a gAd-overexpressed apoE-deficient (gAd Tg apoE-/-:b) mouse.
Fig. 20 shows the areas of the foci of arteriosclerosis in an apoE-deficient (apoE-/-) mouse and a gAd-overexpressed apoE-deficient (gAd Tg apoE-/-) mouse (aortic arch (b), descending aorta (c), and their sum (a)).
Fig. 21 shows the results of Oil Red O staining, reaction with anti-Mac3 antibody, and reaction with anti-scavenger receptor A antibody as observed in an apoE-deficient (apoE-/-) mouse and a gAd-overexpressed apoE-deficient (gAd Tg apoE-/-) mouse.

Best Mode for Carrying Out the Invention

[0012] Adiponectin, which is employed in the present invention, has already been cloned (Maeda, K. et al., Biochem. Biophys. Res. Commun. 221, 286-296 (1996), Nakano, Y. et al., J. Biochem. (Tokyo) 120, 802-812 (1996)), and is available through known means. SEQ ID NOs: 1 and 2 show the nucleotide sequence and the amino acid sequence of human adiponectin. Adiponectin is composed of an N-terminal collagen-like sequence (cAd) a C-terminal globular domain (gAd; in SEQ ID NO: 1, amino acid Nos. 114 to 239 or 111 to 242). The C-terminal globular domain (gAd) is preferred as it provides stronger arteriosclerosis preventive and therapeutic effects than full length adiponectin. SEQ ID NOs: 3 and 4 show the nucleotide sequence and the amino acid sequence of mouse adiponectin. The N-terminal collagen-like sequence (cAd) of the mouse adiponectin stretches from 45 to 109 (amino acid No.), and the C-terminal globular domain (gAd) stretches from 110 to 247 (amino acid No.). According to the present invention, not only proteins comprising the amino acid sequence of any of SEQ ID NOs: 1 to 4 or an amino acid sequence having the gAd domain, but also a protein comprising a modified amino acid sequence derived from substitution, deletion, or addition of one or more amino acid residues of any of these amino acid sequences may be employed, so long as it provides an effect as exhibited by adiponectin. Examples of such mutated proteins include those having 80% or higher homology, preferably 90% or higher homology, to any of the amino acid sequences of SEQ ID NOs: 1 to 4 or an amino acid sequence

including the gAd domain.

[0013] Examples of the gene which is employed in the present invention include the genes coding for adiponectin (i.e., SEQ ID NOs: 1 and 3) and a gene coding for gAd. Also, there may be employed a gene having a nucleotide sequence capable of hybridizing with any of these genes under stringent conditions.

[0014] Adiponectin or a polypeptide which forms a portion of adiponectin (including gAd) may be isolated from cells containing the same. However, since a gene coding for adiponectin has already been cloned, the adiponectin or the polypeptide may be prepared through a DNA recombinant technique; i.e., making use of transformant cells created by use of expression vectors produced through use of the gene.

[0015] As will be described hereinbelow, adiponectin-deficient mice exhibit high levels of neutral fat in blood, but their cholesterol levels are comparable to those of wild-type mice. Moreover, adiponectin-deficient mice, representing an arteriosclerosis model, exhibited intima thickening which was twice the thickness as observed in wild-type mice. In contrast, when apoE-deficient mice, which represent a spontaneous atherosclerosis model, are caused to over-express gAd, they exhibit a significant reduction in arteriosclerotic area, preventing development of arteriosclerosis. However, overexpression of gAd induced in apoE-deficient mice only insignificantly affect general risk factors for arteriosclerosis, such as body weight and blood sugar, and free fatty acid, neutral fat, and total cholesterol in blood. On the other hand, over-expression of gAd induced in apoE-deficient mice was found to exhibit a lowered expression of scavenger receptor A in arterial walls. Scavenger receptor A is a receptor which, when macrophages engulf modified LDL, binds to the modified LDL on the surface of a cell, and is known to play a key role as a receptor which triggers the onset of arteriosclerosis.

[0016] Accordingly, adiponectin, gAd, or a gene coding for adiponectin or gAd is useful as a down-regulator of scavenger receptor A expression, or as a drug for preventing or treating arteriosclerosis. In particular, gAd or a gene encoding gAd is very useful in that it exhibits a more potent down-regulating effect on expression of a scavenger receptor as compared with adiponectin, and stronger preventive or therapeutic effect.

[0017] For administering the drug of the present invention to a mammal including a human, pharmaceutical compositions of a variety of dosage forms may be produced through incorporation of a pharmacologically acceptable carrier to any of the aforementioned active ingredients. Among such dosage forms, preparations for injection are preferred. Examples of the pharmacologically acceptable carrier include distilled water, a solubilizer, a stabilizer, an emulsifier, and a buffer. The dose of any of the drugs may differ depending on the condition of the disease, sex, body weight, etc., and may range from 0.1 μg to 10 mg/day or thereabouts, as reduced to the amount of adiponectin or gAd.

Examples

[0018] The present invention will next be described in more detail by way of examples, which should not construed as limiting the invention thereto.

A. Methods

(1) Preparation of knockout mice

[0019] Screening of a 129/Sv mouse genomic library was performed using adiponectin cDNA as a probe, whereby a plurality of clones harboring a gene encoding adiponectin were obtained. A targeting vector was constructed, in which the region stretching from the translation initiation site to the translation termination site had been replaced by a neomycin-resistant gene. ES cells were transfected with the resultant targeting vector. Screening was performed through Southern blotting, whereby homologous recombinants of 5 clones were confirmed. Chimeric mice were created by means of microinjection, and the mice were crossbred with BI/6 to thereby produce F1, and then F2.

[0020] Briefly, an adiponectin-gene-deficient mouse was produced through homologous recombination as shown in Fig. 1. With an aim to knock out the mouse adiponectin gene, a targeting vector in which exons 2 and 3 that encode adiponectin were replaced with a neo resistant gene was prepared. Separate 5 homologous recombinant clones were confirmed through Southern blotting (Fig. 2). From ES cells having 129/Sv as a background, chimeric mice were produced, and in order to create a hetero-deficient mouse, they were cross-bred with BI/6. The genotype was confirmed through Southern blotting (Fig. 3).

(2) Insulin tolerance test

[0021] Human insulin was intraperitoneally administered to test mice in an amount of 0.7 mU per gram (body weight), and the mice were fasted during the tolerance test. The blood was collected from the tail vein, and blood sugar level was measured by means of a Glutest Ace (registered trademark, product of Sanwa Kagaku Kenkyusho Co., Ltd.).

(3) Glucose tolerance test

[0022] Glucose was perorally administered to test mice in an amount of 1.5 mg per gram (body weight). Prior to the administration, the mice had been fasted for at least 16 hours. The blood was collected from the fundus vein, and blood sugar level and insulin level were measured by means of a Glutest Ace (registered trademark, product of Sanwa Kagaku Kenkyusho Co., Ltd.) and a rat insulin RIA kit (product of Amersham Pharmacia Biotech), respectively.

(4) Measurement of blood lipid level

[0023] After the test mice were fasted for 16 hours, levels of free fatty acid, neutral fat, and total cholesterol, all in blood, were measured by means of a NEFAC-test, a TGL-type, and a Tchol E-type (product of Wako), respectively.

(5) Measurement of blood leptin level and blood adiponectin level

[0024] After the mice were fasted for 16 hours, levels of leptin and adiponectin, both in blood, were measured by means of a Quintikine M kit (product of R&D) and an adiponectin RIA kit (product of Linco), respectively.

(6) Creation of a thick vascular intima model through cuff placement

[0025] A 2.0-mm polyethylene tube (PE-50) was placed in the femoral artery. When two weeks had passed, the artery was press-fixed with formalin, and removed together with the opposite-side, uncuffed artery, which served as a control artery. Each of the thus-removed blood vessels was sliced to obtain continuous ring-shaped specimens, each having a length of 10 mm. Ten specimens were taken and HE staining was performed. The inner diameter of the blood vessel, the thickness of the intima, and the thickness of the media were measured, and intima/media ratio was calculated.

(7) Preparation of gAd-overexpressed mice

[0026] According to the method described in Diabetes 48, 1822-1829 (1999), a fused gene containing a human SAP promoter and mouse gAd cDNA was prepared. Purified Hind III-XhoI fragments were microinjected to pronuclei of fertilized ova of C57BL6 mice (product of Clea Japan, Inc.). Tail DNA samples obtained from the resultant transgenic mice were subjected to Southern blotting through use of a gAd cDNA probe for the Bgl II/Hinc II site of gAd, whereby gAd overexpression of the transgenic mice was confirmed.

(8) Production of gAd-overexpressing apoE-deficient mice

[0027] gAd-overexpressing apoE-deficient mice were crossbred, to thereby produce gAd-overexpressing apoE-hetero-deficient mice. The resultant mice were crossed further with apoE-deficient mice, to thereby create apoE-deficient mice exhibiting over-expression of gAd.

(9) Measurement of blood sugar level and lipid level

[0028] Mice were fed until they were full, and their blood sugar level and levels, in blood, of free fatty acid, neutral fat, and total cholesterol were measured by means of a Glutest Ace (registered trademark, product of Sanwa Kagaku Kenkyusyo Co., Ltd.), an NEFA C-test, a TGL-type, and a Tchol E-type (Products of Wako), respectively.

(10) Evaluation of the size of arteriosclerotic foci

[0029] From each of gAd-overexpressed apoE-deficient mice (4 months old) and control apoE-deficient mice, the aortic arch and the descending aorta were removed, fixed with formalin, and then subjected to staining with Sudan IV. The arteriosclerotic foci were evaluated in terms of their size.

(11) Evaluation in terms of buildup of cholesterol ester, expression level of scavenger receptor A, and macrophage accumulation

[0030] Frozen samples of continuous ring-shaped specimens of the annulus portion of the aorta were prepared. Ten such samples were subjected to immunostaining by use of Oil Red O, anti-scavenger receptor A antibody, or anti-Mac3 antibody (a macrophage-specific marker), whereby buildup of cholesterol ester, expression level of scavenger receptor

A, and macrophage accumulation were evaluated, respectively.

B. Results

(1) Mouse-adiponectin-gene-deficient mice

[0031] Through Northern blotting of white adipose tissue, the expression level of adiponectin in the hetero-deficient mice was found to be reduced by about 60%, and the homo-deficient mice were found to exhibit completely no adiponectin expression (Fig. 4). Indeed, when blood adiponectin level was measured in the hetero-deficient mice, the magnitude of reduction was found to be about 60%, and the level in the hetero-deficient mice was found to be lower than the undetectable level (Fig. 5). With respect to the blood leptin level, no difference was observed (Fig. 6).

(2) Insulin resistance of mouse-adiponectin-gene-deficient mice

[0032] In three groups of 6-week-old mice; i.e., wild-type group, hetero-deficient (adipo +/-) group, and homo-deficient (adipo -/-) group, there was no difference in terms of body weight (Fig. 7). The 6-week-old wild-type mice and hetero-defective mice of the same age were subjected to an insulin tolerance test, to thereby check their insulin sensitivity. The degree of reduction in blood sugar level in response to exogenous insulin was statistically significantly low in the hetero-deficient mice, proving that the hetero-deficient mice had insulin resistance (Fig. 8).

[0033] Next, a glucose tolerance test was performed. No difference was observed between the two groups of wild-type mice and hetero-deficient mice in terms of blood sugar or insulin level (Fig. 9). However, as compared with the wild-type mice, the hetero-deficient mice, after having been loaded with 10-week high fat diet, exhibited a significantly high blood sugar level before and after loading with glucose, though the body weight remained in a similar level (Fig. 10).

[0034] Afterwards, analysis on the homo-deficient mice was performed.

[0035] An insulin tolerance test performed on 6-week-old wild-type mice and homo-deficient mice of the same age. As compared with the wild-type mice or the hetero-deficient mice, the degree of reduction in blood sugar level in response to exogenous insulin was statistically significantly low in the homo-deficient mice, proving that the homo-deficient mice had insulin resistance higher than the corresponding levels of the wild-type mice and homo-deficient mice (Fig. 11).

[0036] Next, a glucose tolerance test was performed. In both stages of during fasting and after glucose loading, the homo-deficient mice exhibited blood sugar levels higher than the case of wild-type mice. This substantiates that homo-deficient mice had slightly impaired glucose tolerance in addition to insulin resistance (Fig. 12). Before administration and 30 minutes after administration, no difference was observed between the wild-type group and the homo-deficient group in terms of the insulin levels before and after glucose loading. However, the homo-deficient mice showed a somewhat low insulin level at 15 min (Fig. 12).

(3) Blood neutral fat level in adiponectin homo-deficient mice

[0037] In order to check the effect of adiponectin on lipid metabolism, levels, in blood, of free fatty acid (FFA), neutral fat (TG), and total cholesterol (TC) were determined in wild-type, hetero-deficient, and homo-deficient mice (Figs. 13 and 14). The hetero-deficient mice did not show any difference in level of any of the three test items as compared with the wild-type mice (Fig. 13). However, the homo-deficient mice showed significantly higher blood neutral fat levels than the wild-type mice (Fig. 14).

(4) Thickening of intima in cuff-injured models of mouse adiponectin hetero-deficient mice

[0038] In order to investigate-the effect of adiponectin on arteriosclerosis, the degree of intimal thickening induced by cuff placement was measured in the wild-type mice and the hetero-deficient mice for comparison therebetween. No difference was observed between the two groups in terms of the vascular inner diameter after cuff-induced injury was created (Fig. 15). When 2 weeks had elapsed after creation of cuff injury, the hetero-deficient mice showed about 1.8 times the thickness of the intima of the wild-type mice (Fig. 16). However, no difference was observed between the two groups in terms of the thickness of the media (Fig. 17). The intima/media ratio of the hetero-deficient group exhibited a ratio about two-fold that of the wild-type mice (Fig. 18).

(5) Prevention of the onset of arteriosclerosis in gAd-overexpressing apoE-deficient mice

[0039] ApoE-deficient mice, which represent a spontaneous arteriosclerosis model, were caused to overexpress gAd and studied whether or not onset of arteriosclerosis was prevented. The results are shown in Figs. 19 and 20. In

Fig. 19, "a" shows the results of Sudan IV staining of aorta samples from apoE-deficient mice, and "b" shows the results of Sudan IV staining of aorta samples from gAd-overexpressed apoE-deficient mice. As is evident from the comparison between "a" and "b," gAd-overexpressed apoE-deficient mice clearly show a reduction in the incidence of arteriosclerotic foci. Fig. 20 shows comparison with respect to the area of arteriosclerotic foci. Fig. 20 shows that over-expression of gAd caused significant reduction in the area of arteriosclerotic foci, which are stained with Sudan IV, in any case of aortic arch (b), descending aorta (c), the sum of the mentioned two cases (a), indicating arresting of the onset of arteriosclerosis.

(6) Effect of gAd overexpression on arteriosclerosis risk factors in apoE-deficient mice on a normal diet

[0040] The body weight, blood sugar level, and levels, in blood, of free fatty acid, neutral fat, and total cholesterol of gAd-overexpressed apoE-deficient mice on a normal diet are shown in Table 1.

Table 1

	Mouse		
	apoE-/-	gAd Tg apoE-/-	Statistical significance
Body weight(g)	29.8±1.2	29.9±1.5	none
Plasma glucose level (mg/dl)	145±4	152±8	none
Serum total cholesterol level (mg/dl)	541±49	509±32	none
Serum triglyceride level (mg/dl)	127±52	104±24	none
Serum free fatty acid level (mEq/L)	0.53±0.08	0.57±0.04	none
Mean ± s.e. (n=5)			

[0041] As shown in Table 1, when apoE-deficient mice on a normal diet were caused to over-express gAd, no significant effect was exerted on arteriosclerosis risk factors such as body weight and blood sugar, and free fatty acid, neutral fat, and total cholesterol in blood. This suggests that gAd possibly acts on vascular walls or macrophages directly, to thereby exhibit anti-arteriosclerotic activity.

(7) Mechanism of arresting onset of arteriosclerosis by gAd

[0042] With an aim to elucidate the mechanism of the interaction between gAd and vascular walls or macrophages, frozen samples of continuous ring-shaped slices of annulus portion of the aorta were subjected to immunostaining by use of Oil Red O, anti-scavenger receptor A antibody, and a macrophage-specific marker; i.e., anti-Mac3 antibody. As a result, as shown in Fig. 21, over-expression of gAd, though having no significant impact on accumulation of macrophages, were found to reduce the expression level of scavenger receptor A, suppress buildup of lipids in macrophages, and arrest the onset of arteriosclerosis.

Industrial Applicability

[0043] The present invention provides a preventive or therapeutic agent capable of directly preventing intimal thickening, which constitutes an essential feature of arteriosclerosis, wherein this effect can be attained through arresting the onset and development of arteriosclerosis by reducing the expression level of scavenger receptor A in arterial walls and preventing lipid buildup in macrophages.

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His Pro Gly His Asn Gly Thr Pro Gly Arg Asp Gly Arg Asp Gly Thr
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        195                 200                 205
Glu Val Gly Asp Gln Val Trp Leu Gln Val Tyr Gly Asp Gly Asp His
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225                 230                 235                 240
Leu Leu Tyr His Asp Thr Asn
                245
```

Claims

1. A drug for preventing or treating arteriosclerosis, containing, as an active ingredient thereof, a C-terminal globular domain of adiponectin, adiponectin, or a gene encoding the domain or adiponectin.

2. The drug for preventing or treating arteriosclerosis as claimed in claim 1, wherein the active ingredient is a C-terminal globular domain of adiponectin or a gene encoding the domain.

3. The drug for preventing or treating arteriosclerosis as claimed in claim 1 or 2, further containing a pharmacologically acceptable carrier.

4. A scavenger receptor A expression down-regulator, containing, as an active ingredient thereof, a C-terminal glob-

ular domain of adiponectin, adiponectin, or a gene encoding the domain or adiponectin.

5. The scavenger receptor A expression down-regulator as claimed in claim 4, wherein the active ingredient is a C-terminal globular domain of adiponectin or a gene encoding the domain.

6. Use of a C-terminal globular domain of adiponectin, adiponectin, or a gene encoding the domain or adiponectin in manufacture of a drug for preventing or treating arteriosclerosis.

7. The use as claimed in claim 6, wherein the component is a C-terminal globular domain of adiponectin or a gene encoding the domain.

8. Use of a C-terminal globular domain of adiponectin, adiponectin, or a gene encoding the domain or adiponectin in manufacture of a scavenger receptor A expression down-regulator.

9. The use as claimed in claim 6, wherein the component is a C-terminal globular domain of adiponectin or a gene encoding the domain.

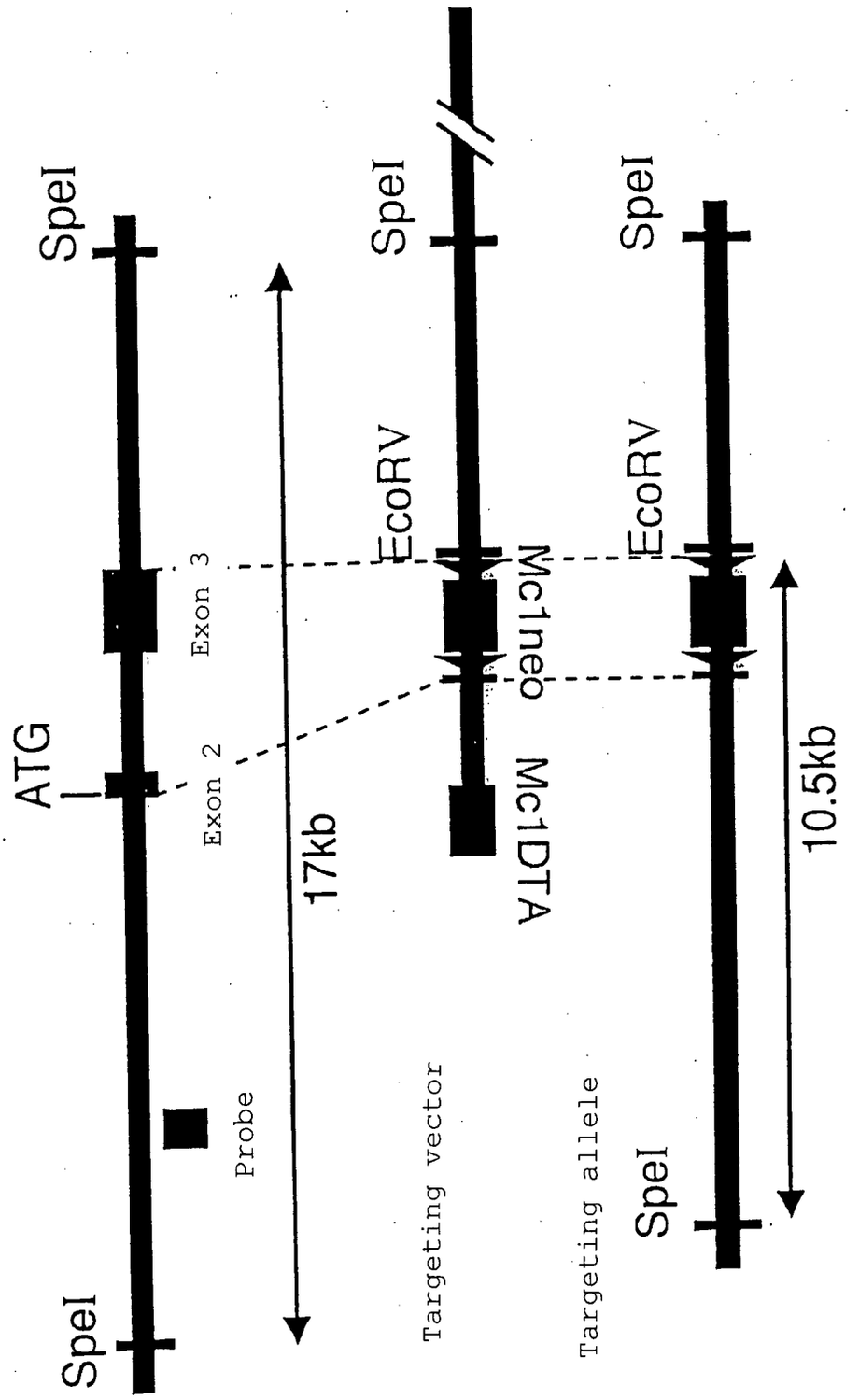
10. A method for treating arteriosclerosis, comprising administering a C-terminal globular domain of adiponectin, adiponectin, or a gene encoding the domain or adiponectin in an effective amount to a subject in need thereof.

11. The method for treating arteriosclerosis as claimed in claim 10, comprising administering a C-terminal globular domain of adiponectin or a gene encoding the domain.

12. A method for down-regulating expression of scavenger receptor A in a subject, comprising administering a C-terminal globular domain of adiponectin, adiponectin, or a gene encoding the domain or adiponectin in an effective amount to the subject.

13. The method for down-regulating expression of scavenger receptor A as claimed in claim 12, comprising administering a C-terminal globular domain of adiponectin or a gene encoding the domain.

Fig. 1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SpeI
ATG
Exon 3
Exon 2
Probe
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Targeting vector
SpeI
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SpeI
10.5kb
Targeting allele

Fig. 2

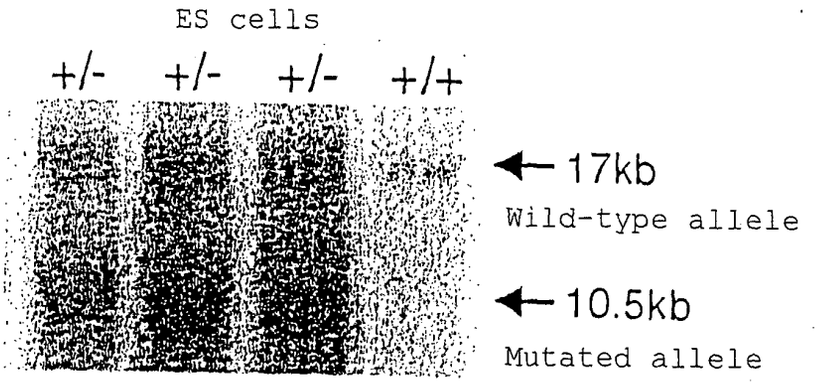
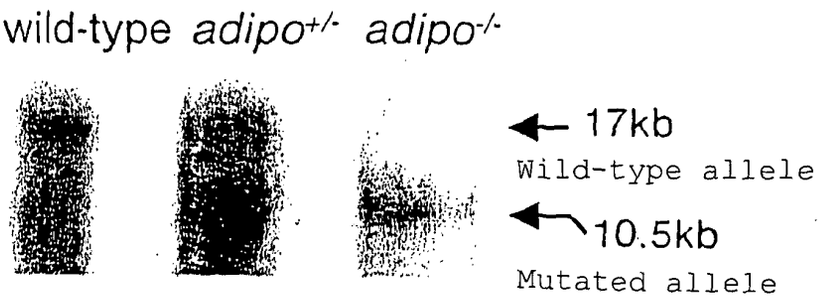


Fig. 3



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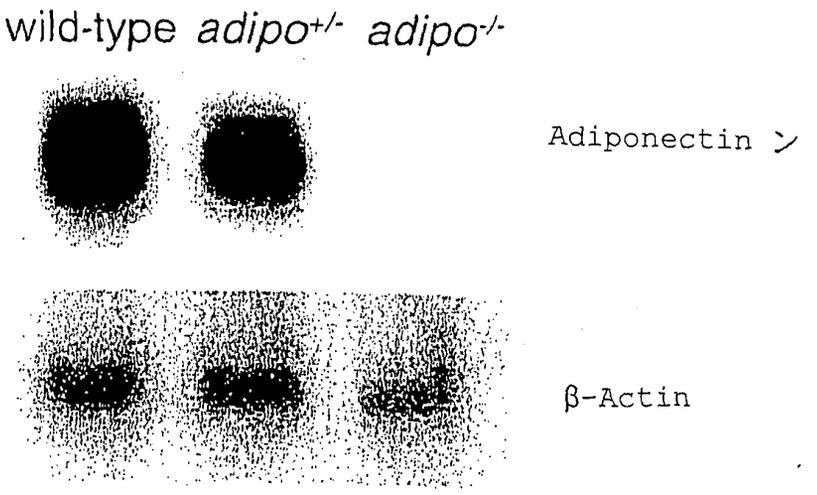
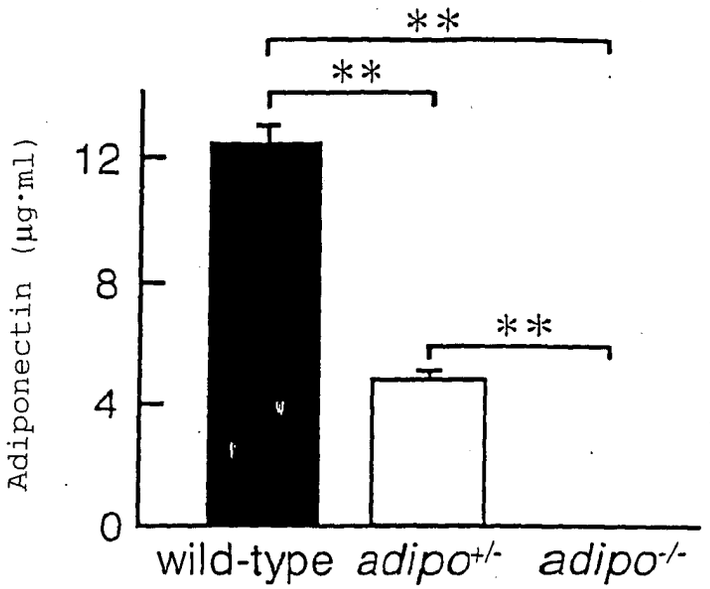


Fig. 5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**
**
Adiponectin (μg·ml)
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adipo+/-
adipo-/-

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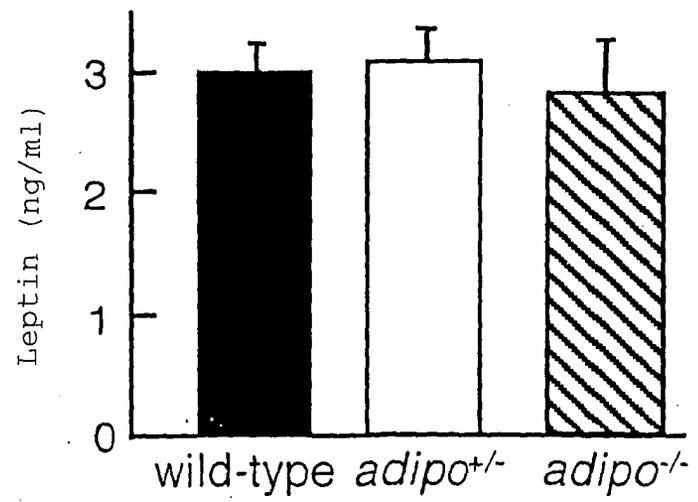
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adipo-/-

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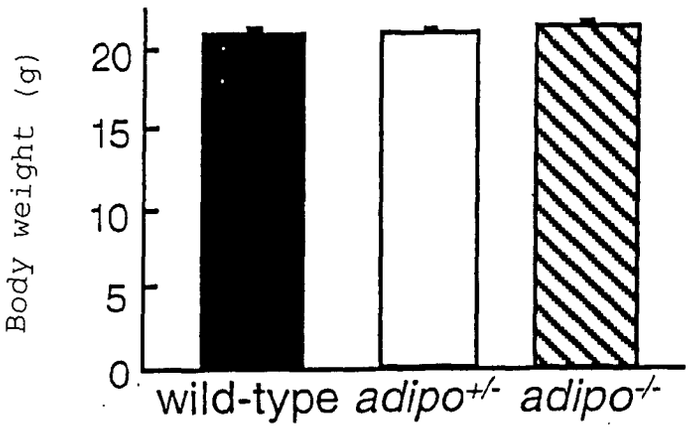
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15
10
5
0
wild-type
adipo+/-
adipo-/-

Fig. 8

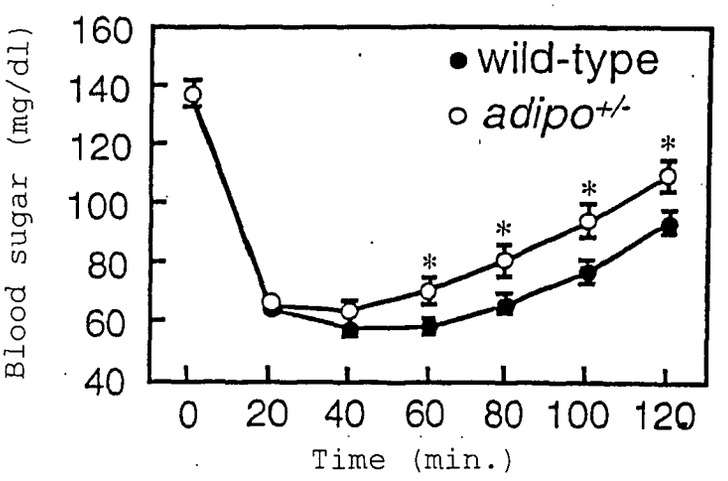
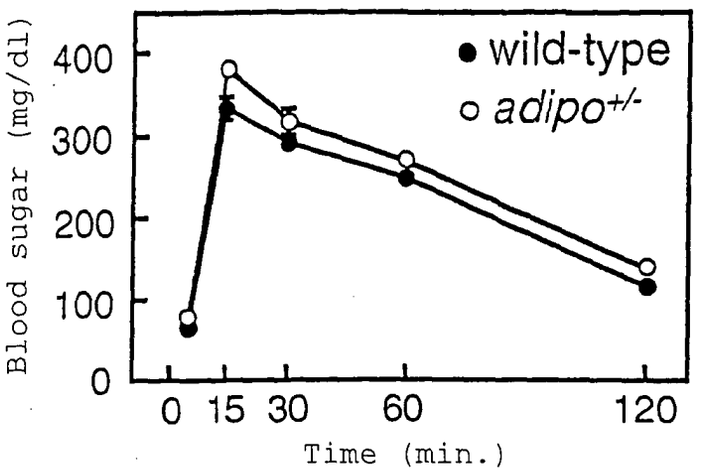

Blood sugar (mg/dl)
160
140
120
100
80
60
40
● wild-type
○ adipo+/-
*
*
*
*
0
20
40
60
80
100
120
Time (min.)

Fig. 9

(1)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300
200
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0
0 15 30 60 120
Time (min.)
● wild-type
○ adipo+/-

(2)

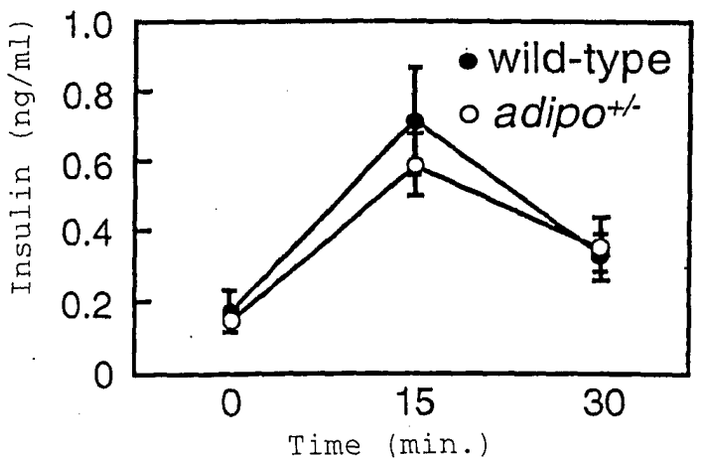
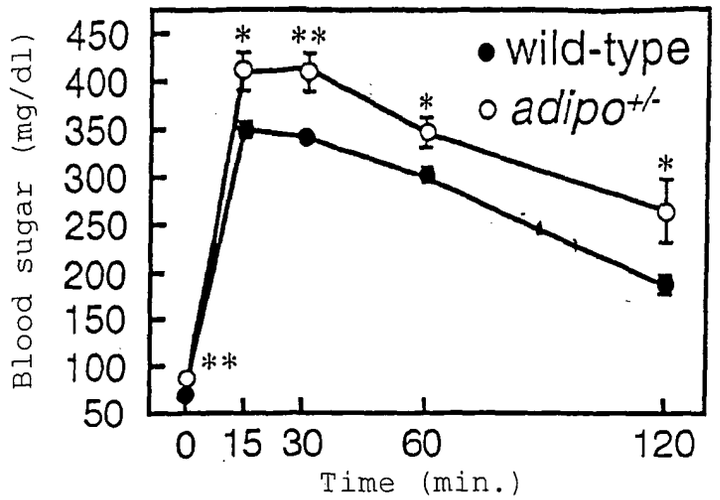

Insulin (ng/ml)
1.0
0.8
0.6
0.4
0.2
0
0 15 30
Time (min.)
● wild-type
○ adipo+/-

Fig. 10

(1)



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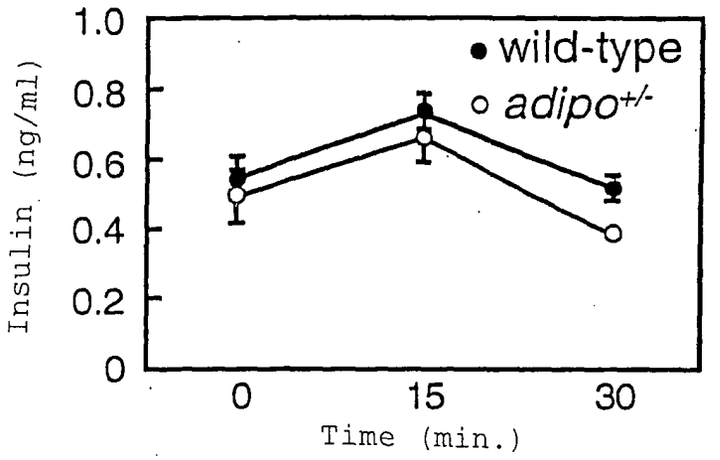

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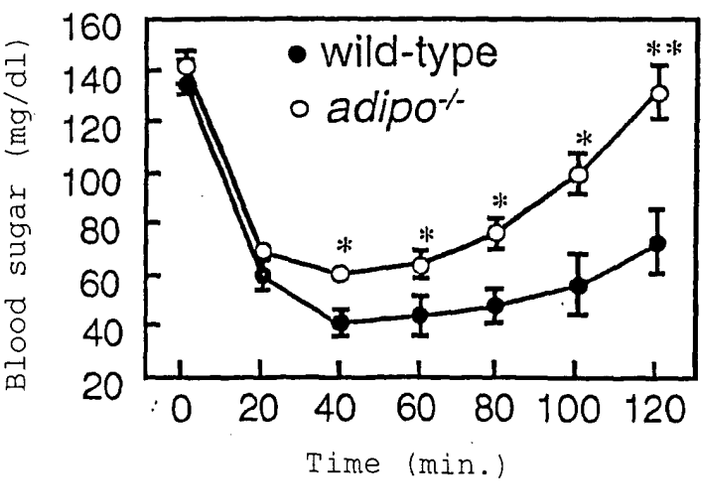
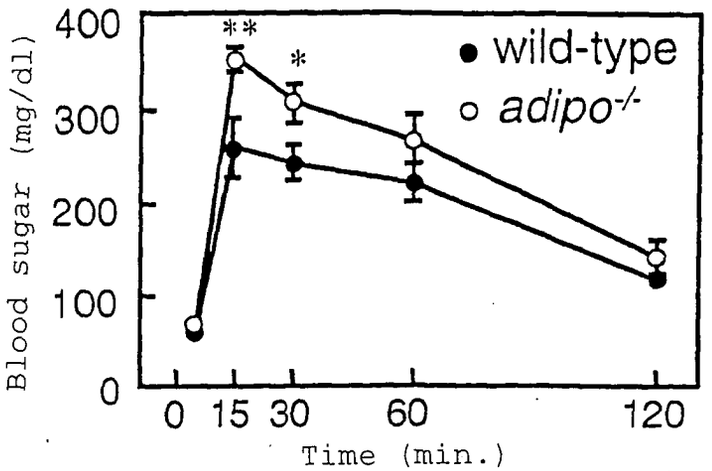


Fig. 12

(1)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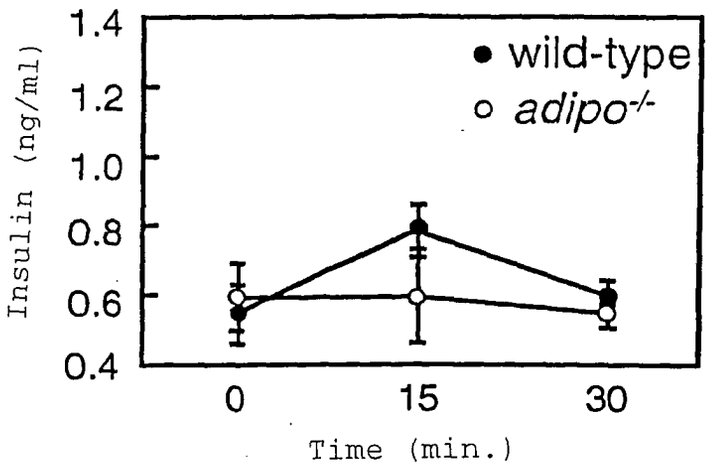
200
100
0
**
*
● wild-type
○ adipo-/-
0 15 30 60 120
Time (min.)

(2)


Insulin (ng/ml)
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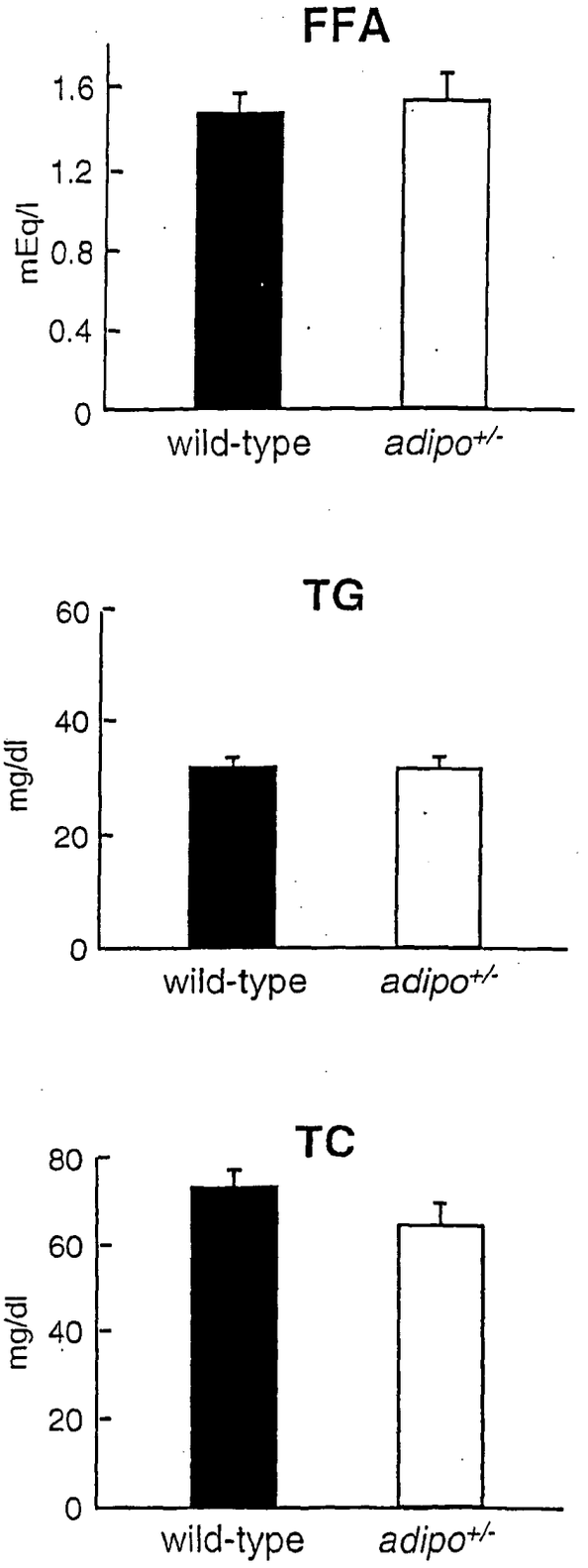
1.2
1.0
0.8
0.6
0.4
● wild-type
○ adipo-/-
0 15 30
Time (min.)

Fig. 13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1.2
0.8
0.4
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mEq/l
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adipo+/-
TG
60
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wild-type
adipo+/-
TC
80
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40
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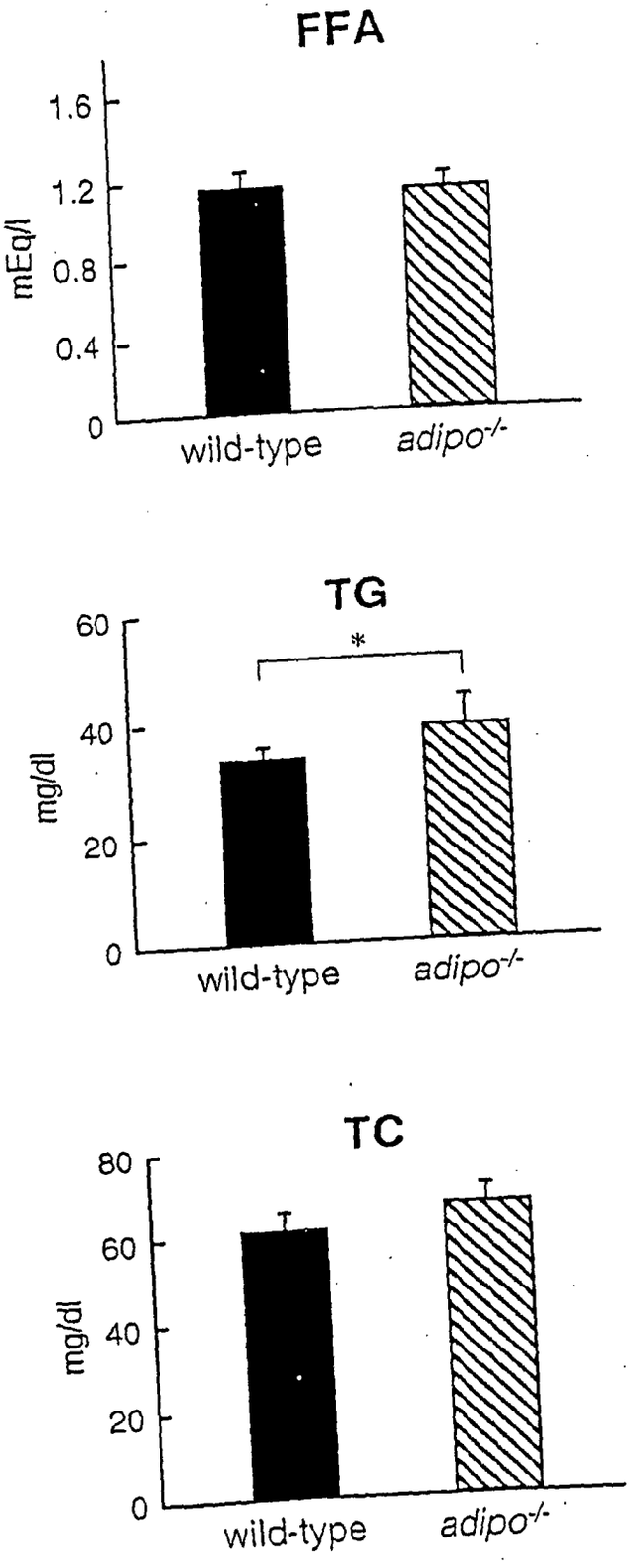
0
mg/dl
wild-type
adipo+/-

Fig. 14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1.2
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0.4
0
mEq/l
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adipo-/-
TG
*
60
40
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adipo-/-
TC
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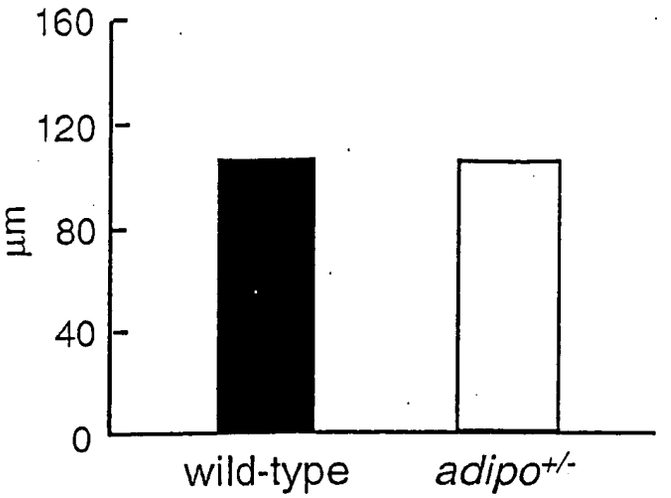
60
40
20
0
mg/dl
wild-type
adipo-/-

Fig. 15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120
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40
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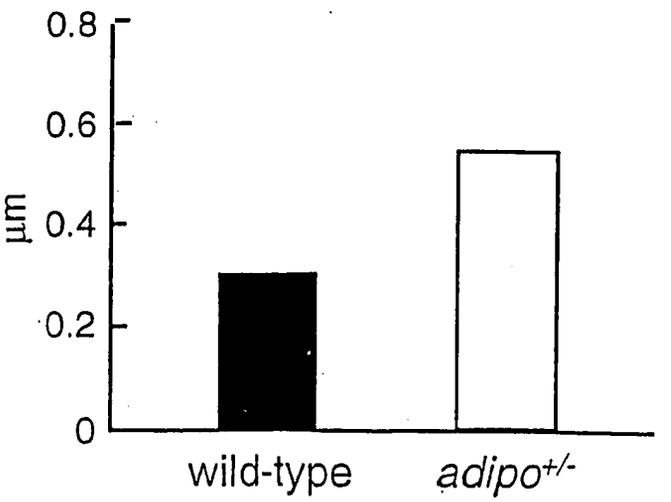
µm
wild-type
adipo+/-

Fig. 16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0.6
0.4
0.2
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µm
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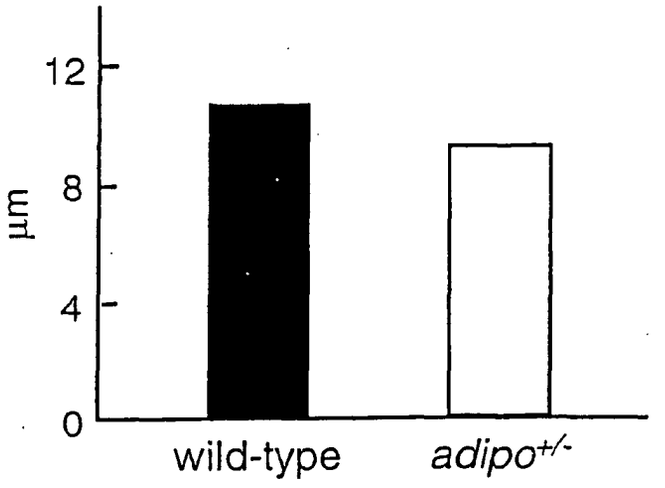
adipo+/-

Fig. 17



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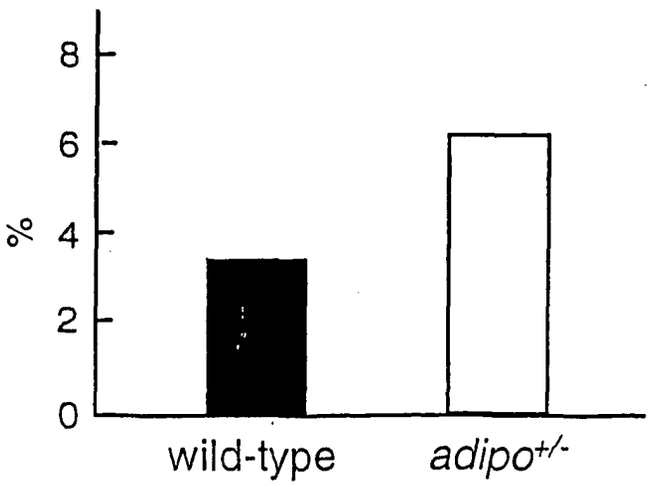

Fig. 19

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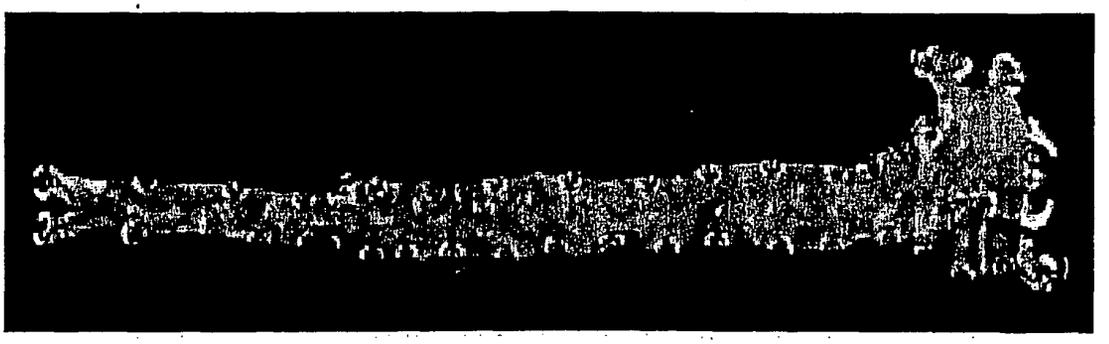


b gAdTg apoE-/-

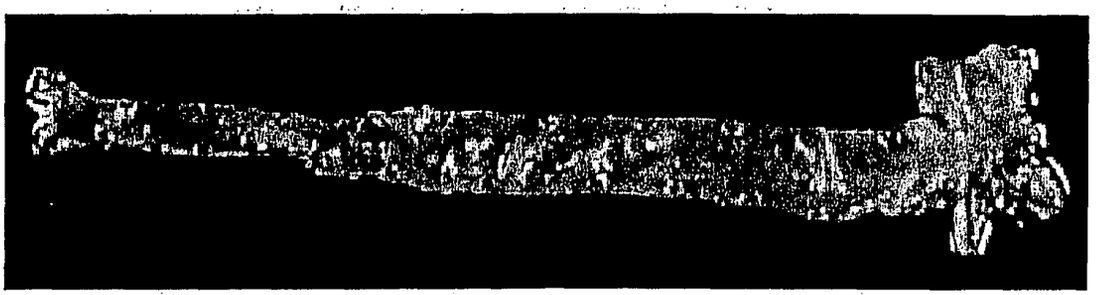
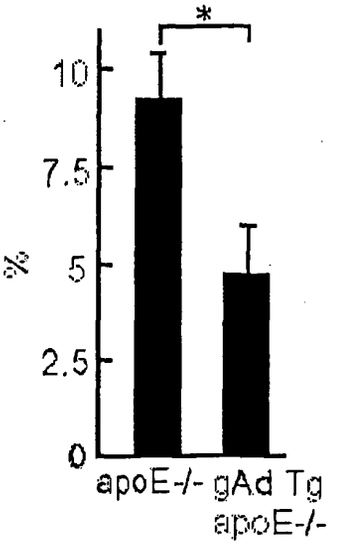
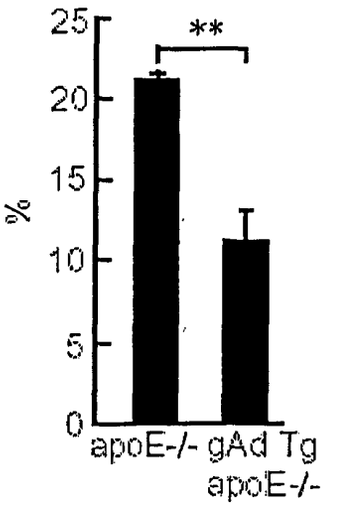


Fig. 20

a (Sum)



b (Aorta)



c (Descending aorta)

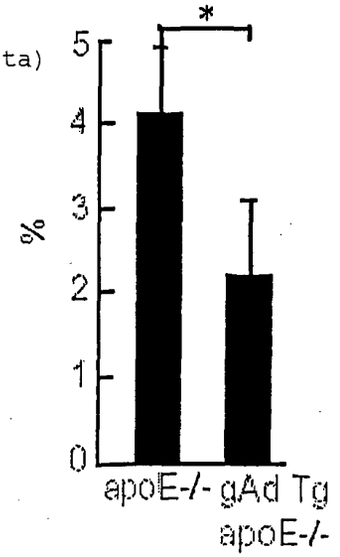
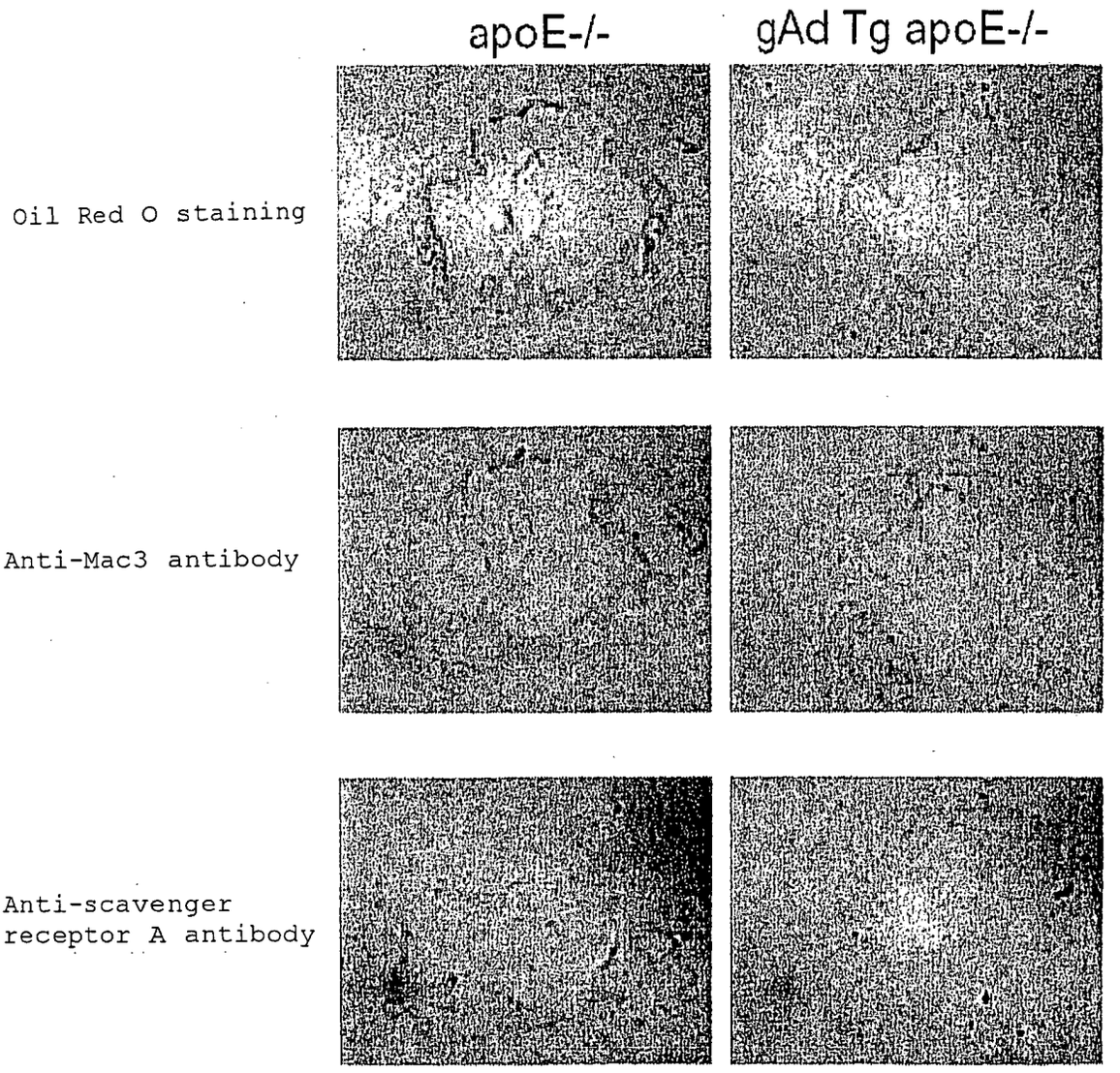


Fig. 21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Oil Red O staining
Anti-Mac3 antibody
Anti-scavenger receptor A antibody

INTERNATIONAL SEARCH REPORT

International application No.
PCT/JP03/06518

A. CLASSIFICATION OF SUBJECT MATTER
Int.Cl[7] A61K38/17, 48/00, A61P9/10, 43/00

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)
Int.Cl[7] A61K38/17, 48/00, A61P9/10, 43/00

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

Jitsuyo Shinan Koho	1926-1992	Toroku Jitsuyo Shinan Koho	1994-1996
Kokai Jitsuyo Shinan Koho	1971-1992	Jitsuyo Shinan Toroku Koho	1996-2003

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
CA(STN), MEDLINE(STN), BIOSIS(STN), EMBASE(STN)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	OUCHI, Noriyuki et al., Adipocyte-derived plasma protein, adiponectin, suppresses lipid accumulation and class A scavenger receptor expression in human monocyte-derived macrohages, Circulation, 2001, Vol.103, No.8, pages 1057 to 1063	1-9
X	Database BIOSIS on STN, AN 2000:26346, OUCHI, Noriyuki et al., A novel adipocyte-derived plasma protein, adiponectin, suppresses scavenger receptor expression in human monocyte-derived macrophages., Circulation, 1999, Vol,110, No.18 SUPPL., pages I.751.Meeting Info. : 72nd Scientific Sessions of the America Heart Association	1-9

☒ Further documents are listed in the continuation of Box C. ☐ See patent family annex.

* Special categories of cited documents:
"A" document defining the general state of the art which is not considered to be of particular relevance
"E" earlier document but published on or after the international filing date
"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)
"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
"T" 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
"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
"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
"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
11 July, 2003 (11.07.03)	22 July, 2003 (22.07.03)
Name and mailing address of the ISA/ Japanese Patent Office	Authorized officer
Facsimile No.	Telephone No.

Form PCT/ISA/210 (second sheet) (July 1998)

INTERNATIONAL SEARCH REPORT

International application No.
PCT/JP03/06518

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	Database BIOSIS on STN, AN 2001:27386, KIHARA, Shinji et al., Anti-atherogenic property of adipocyte-derived plasma protein, adiponectin., Journal of Molecular and Cellular Cardiology, 2000, Vol.32, No., ppA93.print.Meeting Info : XVII Annual Meeting of the International Society for Heart Research	1-9
A	MAEDA, Kazuhisa et al., cDNA cloning and expression of a novel adipose specific collagen-like fector, apM1(adipose most abundant gene transcript 1), Biochemical and Biophysical Research Communications, 1996, Vol.221, No.2, pages 286 to 289	1-9
P,X	OKAMOTO, Yoshihisa et al., Adiponectin Reduces Atherosclerosis in Apolipoprotein E-Deficiect Mice, Circulation, 26 November, 2002 (26.11.02), Vol.106, No.22, pages 2767 to 2770	1-9
P,X	WO 02/061076 A1 (Mochida Pharmaceutical Co., Ltd.), 08 August, 2002 (08.08.02), Full text (Family: none)	1-9

Form PCT/ISA/210 (continuation of second sheet) (July 1998)

INTERNATIONAL SEARCH REPORT

International application No.
PCT/JP03/06518

Box I Observations where certain claims were found unsearchable (Continuation of item 2 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. [X] Claims Nos.: 10-13
because they relate to subject matter not required to be searched by this Authority, namely:
Claims 10 to 13 involve methods for treatment of the human body by surgery or therapy and thus relate to a subject matter which this International Searching Authority is not required, under the provisions of Article 17(2)(a)(i) of the PCT and Rule 39.1(iv) of the Regulations under the PCT, to search.

2. [ ] Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. [ ] Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 3 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. [ ] As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.

2. [ ] As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. [ ] As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. [ ] No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

[ ] The additional search fees were accompanied by the applicant's protest.

[ ] No protest accompanied the payment of additional search fees.

Form PCT/ISA/210 (continuation of first sheet (1)) (July 1998)