of SNPs for which the association between SNPs and type 2 diabetes was investigated in the present study). We further genotyped SNP15 in *ADIPOR2*, which showed a tendency towards an association with HOMA (p=0.04), in a second panel to test reproducibility. The statistical analyses, except for those for haplotype estimation, were performed using JUMP for Windows, version 4.00 (SAS Institute, Cary, NC, USA).

Haplotype analysis Tagged SNPs were selected for haplotype analysis using HaploBlockFinder software (http://www.cgi.uc.edu/cgi-bin/kzhang/haploBlockFinder.cgi, last accessed in April 2005). The tagged SNPs consisted of a minimal set of SNPs that were uniquely distinguishable from at least 90% of the common haplotypes. After selecting the tagged SNPs, the frequency of each haplotype was estimated and differences in haplotype frequencies between non-diabetic and diabetic subjects were assessed

Table 1 Comparison of genotypic and allelic distribution of SNPs in *ADIPOR1* between type 2 diabetic subjects and non-diabetic subjects

SNP	Rs number	Position (kb)	Genotype, *n* (%)			*p* value	Allele, *n* (%)		*p* value
SNP1	6666089	−8.505	11	12	22		1	2	
NDM			45 (23.4%)	101 (52.6%)	46 (24.0%)		191 (49.7%)	193 (50.3%)	
T2DM			49 (25.5%)	100 (52.1%)	43 (22.4%)	0.871	198 (51.6%)	186 (48.4%)	0.613
SNP6	12039275	−5.692	11	12	22		1	2	
NDM			78 (40.6%)	90 (46.9%)	24 (12.5%)		246 (64.1%)	138 (35.9%)	
T2DM			79 (41.1%)	87 (45.3%)	26 (13.5%)	0.934	245 (63.8%)	139 (36.2%)	0.94
SNP7		−4.612	11	12	22		1	2	
NDM			146 (76.0%)	42 (21.9%)	4 (2.1%)		334 (87.0%)	50 (13.0%)	
T2DM			147 (76.6%)	42 (21.9%)	3 (1.6%)	0.929	336 (87.5%)	48 (12.5%)	0.829
SNP8		−4.519	11	12	22		1	2	
NDM			155 (81.2%)	34 (17.8%)	2 (1.0%)		344 (90.1%)	38 (9.9%)	
T2DM			151 (78.6%)	38 (19.8%)	3 (1.6%)	0.790	340 (88.5%)	44 (11.5%)	0.499
SNP9		−2.635	11	12	22		1	2	
NDM			165 (85.9%)	27 (14.1%)	0 (0.0%)		357 (93.0%)	27 (7.0%)	
T2DM			157 (81.8%)	34 (17.7%)	1 (0.5%)	0.368	348 (90.6%)	36 (9.4%)	0.237
SNP12	2275738	−0.106	11	12	22		1	2	
NDM			132 (68.8%)	55 (28.6%)	5 (2.6%)		319 (83.1%)	65 (16.9%)	
T2DM			135 (70.3%)	52 (27.1%)	5 (2.6%)	0.943	322 (83.9%)	62 (16.1%)	0.771
SNP13	2275737	−0.102	11	12	22		1	2	
NDM			134 (69.8%)	54 (28.1%)	4 (2.1%)		322 (83.9%)	62 (16.1%)	
T2DM			144 (75.0%)	44 (22.9%)	4 (2.1%)	0.502	332 (86.5%)	52 (13.5%)	0.31
SNP15		2.850	11	12	22		1	2	
NDM			126 (65.6%)	60 (31.3%)	6 (3.1%)		312 (81.3%)	72 (18.8%)	
T2DM			138 (71.9%)	50 (26.0%)	4 (2.1%)	0.396	326 (84.9%)	58 (15.1%)	0.178
SNP16		3.000	11	12	22		1	2	
NDM			115 (59.9%)	59 (30.7%)	18 (9.4%)		289 (75.3%)	95 (24.7%)	
T2DM			120 (62.5%)	56 (29.2%)	16 (8.3%)	0.860	296 (77.1%)	88 (22.9%)	0.553
SNP18	1342387	5.841	11	12	22		1	2	
NDM			45 (23.4%)	103 (53.6%)	44 (22.9%)		193 (50.3%)	191 (49.7%)	
T2DM			53 (27.6%)	97 (50.5%)	42 (21.9%)	0.644	203 (52.9%)	181 (47.1%)	0.47
SNP19	3737884	6.993	11	12	22		1	2	
NDM			151 (79.1%)	38 (19.9%)	2 (1.0%)		340 (89.0%)	42 (11.0%)	
T2DM			151 (78.6%)	39 (20.3%)	2 (1.0%)	0.995	341 (88.8%)	43 (11.2%)	0.929
SNP20		7.477	11	12	22		1	2	
NDM			75 (39.1%)	93 (48.4%)	24 (12.5%)		243 (63.3%)	141 (36.7%)	
T2DM			67 (34.9%)	98 (51.0%)	27 (14.1%)	0.685	232 (60.4%)	152 (39.6%)	0.414
SNP22	2275736	8.712	11	12	22		1	2	
NDM			136 (70.8%)	53 (27.6%)	3 (1.6%)		325 (84.6%)	59 (15.4%)	
T2DM			139 (72.4%)	49 (25.5%)	4 (2.1%)	0.847	327 (85.2%)	57 (14.8%)	0.840
SNP23	10581	9.879	11	12	22		1	2	
NDM			150 (78.1%)	39 (20.3%)	3 (1.6%)		339 (88.3%)	45 (11.7%)	
T2DM			140 (72.9%)	49 (25.5%)	3 (1.6%)	0.477	329 (85.7%)	55 (14.3%)	0.284

NDM Non-diabetic subjects; *T2DM* type 2 diabetic subjects; *11* major/major; *12* major/minor; *22* minor/minor

Table 2 Comparison of the genotypic and allelic distribution of SNPs in *ADIPOR2* between type 2 diabetic subjects and non-diabetic subjects

SNP	Rs number	Position, kb	Genotype, *n* (%)			*p* value	Allele, *n* (%)		*p* value
SNP2	2058033	−77.219	11	12	22		1	2	
NDM			99 (51.6%)	78 (40.6%)	15 (7.8%)		276 (71.9%)	108 (28.1%)	
T2DM			91 (47.4%)	84 (43.8%)	17 (8.9%)	0.710	266 (69.3%)	118 (30.7%)	0428
SNP4	12579507	−71.725	11	12	22		1	2	
NDM			84 (43.8%)	88 (45.8%)	20 (10.4%)		256 (66.7%)	128 (33.3%)	
T2DM			79 (41.1%)	89 (46.4%)	24 (12.5%)	0.770	247 (64.3%)	137 (35.7%)	0.495
SNP8		−60.706	11	12	22		1	2	
NDM			93 (48.4%)	82 (42.7%)	17 (8.9%)		268 (69.8%)	116 (30.2%)	
T2DM			89 (46.4%)	84 (43.8%)	19 (9.9%)	0.894	262 (68.2%)	122 (31.8%)	0.640
SNP10	10773982	−53.950	11	12	22		1	2	
NDM			52 (27.1%)	98 (51.0%)	42 (21.9%)		202 (52.6%)	182 (47.4%)	
T2DM			62 (32.3%)	96 (50.0%)	34 (17.7%)	0.419	220 (57.3%)	164 (42.7%)	0.192
SNP12	12810020	−52.196	11	12	22		1	2	
NDM			77 (40.1%)	92 (47.9%)	23 (12.0%)		246 (64.1%)	138 (35.9%)	
T2DM			85 (44.3%)	84 (43.8%)	23 (12.0%)	0.684	254 (66.1%)	130 (33.9%)	0.545
SNP13	11061947	−50.799	11	12	22		1	2	
NDM			123 (64.1%)	62 (32.3%)	7 (3.6%)		308 (80.2%)	76 (19.8%)	
T2DM			120 (62.5%)	63 (32.8%)	9 (4.7%)	0.863	303 (78.9%)	81 (21.1%)	0.655
SNP14	11612383	−50.744	11	12	22		1	2	
NDM			56 (29.2%)	96 (50.0%)	40 (20.8%)		208 (54.2%)	176 (45.8%)	
T2DM			51 (26.6%)	101 (52.6%)	40 (20.8%)	0.835	203 (52.9%)	181 (47.1%)	0.718
SNP15	11612726	−47.171	11	12	22		1	2	
NDM			129 (67.2%)	57 (29.7%)	6 (3.1%)		315 (82.0%)	69 (18.0%)	
T2DM			121 (63.0%)	64 (33.3%)	7 (3.6%)	0.691	306 (79.7%)	78 (20.3%)	0.409
SNP17	7976827	−40.160	11	12	22		1	2	
NDM			69 (36.1%)	103 (53.9%)	19 (9.9%)		241 (63.1%)	141 (36.9%)	
T2DM			64 (33.3%)	97 (50.5%)	31 (16.1%)	0.197	225 (58.6%)	159 (41.4%)	0.203
SNP18	12582624	−39.956	11	12	22		1	2	
NDM			71 (37.0%)	94 (49.0%)	27 (14.1%)		236 (61.5%)	148 (38.5%)	
T2DM			78 (40.6%)	90 (46.9%)	24 (12.5%)	0.744	246 (64.1%)	138 (35.9%)	0.455
SNP20		−39.862	11	12	22		1	2	
NDM			63 (32.8%)	99 (51.6%)	30 (15.6%)		225 (58.6%)	159 (41.4%)	
T2DM			57 (29.7%)	99 (51.6%)	36 (18.8%)	0.655	213 (55.5%)	171 (44.5%)	0.382
SNP21	7297509	−33.122	11	12	22		1	2	
NDM			75 (39.1%)	92 (47.9%)	25 (13.0%)		242 (63.0%)	142 (37.0%)	
T2DM			89 (46.4%)	81 (42.2%)	22 (11.5%)	0.352	259 (67.4%)	125 (32.6%)	0.198
SNP22	12818963	−30.884	11	12	22		1	2	
NDM			67 (34.9%)	96 (50.0%)	29 (15.1%)		230 (59.9%)	154 (40.1%)	
T2DM			79 (41.1%)	87 (45.3%)	26 (13.5%)	0.451	245 (63.8%)	139 (36.2%)	0.265
SNP23		−29.409	11	12	22		1	2	
NDM			74 (38.5%)	92 (47.9%)	26 (13.5%)		240 (62.5%)	144 (37.5%)	
T2DM			81 (42.2%)	90 (46.9%)	21 (10.9%)	0.647	252 (65.6%)	132 (34.4%)	0.367
SNP25	10848569	−21.557	11	12	22		1	2	
NDM			48 (25.0%)	101 (52.6%)	43 (22.4%)		197 (51.3%)	187 (48.7%)	
T2DM			52 (27.1%)	95 (49.5%)	45 (23.4%)	0.823	199 (51.8%)	185 (48.2%)	0.885
SNP26	11061974	−15.081	11	12	22		1	2	
NDM			45 (23.4%)	99 (51.6%)	48 (25.0%)		189 (49.2%)	195 (50.8%)	
T2DM			47 (24.5%)	98 (51.0%)	47 (24.5%)	0.971	192 (50.0%)	192 (50.0%)	0.829
SNP27	2068485	−13.571	11	12	22		1	2	
NDM			63 (32.8%)	95 (49.5%)	34 (17.7%)		221 (57.6%)	163 (42.4%)	
T2DM			60 (31.3%)	97 (50.5%)	35 (18.2%)	0.947	21 (56.5%)	167 (43.5%)	0.771
SNP28	7974924	−9.821	11	12	22		1	2	
NDM			56 (29.2%)	96 (50.0%)	40 (20.8%)		208 (54.2%)	176 (45.8%)	

Table 2 (continued)

SNP	Rs number	Position, kb	Genotype, *n* (%)			*p* value	Allele, *n* (%)		*p* value
T2DM			51 (26.6%)	96 (50.0%)	45 (23.4%)	0.768	198 (51.6%)	186 (48.4%)	0.470
SNP29	12831353	−6.333	11	12	22		1	2	
NDM			95 (49.5%)	74 (38.5%)	23 (12.0%)		264 (68.8%)	120 (31.3%)	
T2DM			78 (40.6%)	89 (46.4%)	25 (13.0%)	0.208	245 (63.8%)	139 (36.2%)	0.147
SNP30	12828908	−2.713	11	12	22		1	2	
NDM			50 (26.0%)	94 (49.0%)	48 (25.0%)		194 (50.5%)	190 (49.5%)	
T2DM			36 (18.8%)	96 (50.0%)	60 (31.3%)	0.163	168 (43.8%)	216 (56.3%)	0.060
SNP31	10848571	0.374	11	12	22		1	2	
NDM			104 (54.2%)	76 (39.6%)	12 (6.3%)		284 (74.0%)	100 (26.0%)	
T2DM			91 (47.4%)	83 (43.2%)	18 (9.4%)	0.305	265 (69.0%)	119 (31.0%)	0.129
SNP36	2286385	8.490	11	12	22		1	2	
NDM			56 (29.2%)	98 (51.0%)	38 (19.8%)		210 (54.7%)	174 (45.3%)	
T2DM			48 (25.0%)	100 (52.1%)	44 (22.9%)	0.584	196 (51.0%)	188 (49.0%)	0.312
SNP39		13.274	11	12	22		1	2	
NDM			52 (27.1%)	98 (51.0%)	42 (21.9%)		202 (52.6%)	182 (47.4%)	
T2DM			61 (31.8%)	96 (50.0%)	35 (18.2%)	0.503	218 (56.8%)	166 (43.2%)	0.246
SNP40	12342	14.800	11	12	22		1	2	
NDM			55 (28.6%)	97 (50.5%)	40 (20.8%)		207 (53.9%)	177 (46.1%)	
T2DM			46 (24.0%)	99 (51.6%)	47 (24.5%)	0.500	191 (49.7%)	193 (50.3%)	0.248
SNP41	1044471	14.858	11	12	22		1	2	
NDM			78 (40.6%)	90 (46.9%)	24 (12.5%)		246 (64.1%)	138 (35.9%)	
T2DM			70 (36.5%)	94 (49.0%)	28 (14.6%)	0.661	234 (60.9%)	150 (39.1%)	0.371

NDM Non-diabetic subjects; *T2DM* type 2 diabetic subjects; *11* major/major; *12* major/minor; *22* minor/minor

using a piece of software based on the Expectation Maximisation algorithm (SNPAlyze; Dynacom, Tokyo, Japan). The differences in the haplotype frequencies were then analysed using the chi square test and the permutation test.

Results

We identified a total of 25 SNPs in *ADIPOR1* (Fig. 1a) and 41 SNPs in *ADIPOR2* (Fig. 1b). All of the SNPs that were identified had genotype frequencies that were in Hardy–Weinberg equilibrium in non-diabetic and type 2 diabetic subjects ($p>0.05$). Among these, SNPs with a minor allele frequency higher than 10% were investigated for linkage disequilibrium (LD) in *ADIPOR1* and *ADIPOR2*, and then association with type 2 diabetes and insulin resistance was evaluated. We estimated the degree of LD between pairs of SNPs using an absolute value of D' ($|D'|$). For *ADIPOR1*, the LD extended over 20 kb of the chromosomal region and covered one haplotype block (Fig. 2a). In contrast, the LD in the chromosomal region was less preserved for *ADIPOR2* and was split into three haplotype blocks (Fig. 2b). No differences were observed between the diabetic and non-diabetic subjects in terms of the distribution of the genotypes or alleles of the SNPs in *ADIPOR1* (Table 1) and *ADIPOR2* (Table 2). Only one nominal association was found; this was between SNP15 in *ADIPOR2* and HOMA-IR (11/12/22: 1.27±0.05/1.35±0.08/1.81±0.21, $p=0.04$) (see Tables 3 and 4 of the ESM). When a Bonferroni adjustment was performed (adopted to avoid type 1 errors

Table 3 Distribution of the haplotypes composed of the tagged SNPs in *ADIPOPR1* and *ADIPOR2* in type 2 diabetic subjects and non-diabetic subjects

Haplotype	T2DM	NDM	*p* value	Permutation *p* value
ADIPOR1				
00000	0.4790	0.4521	0.4549	0.3863
10010	0.0500	0.0509	0.9545	0.2367
10001	0.0782	0.0876	0.6367	0.3687
11001	0.1858	0.1934	0.7882	0.6101
11011	0.0600	0.0746	0.4194	0.2117
11111	0.1093	0.0892	0.3521	0.2400
ADIPOR2				
00000	0.5289	0.5260	0.9358	0.6748
11010	0.0653	0.0436	0.1852	0.1443
11011	0.1530	0.1878	0.1992	0.1974
11111	0.1694	0.1626	0.8010	0.6435
ADIPOR2				
00	0.6235	0.6809	0.0949	0.1020
10	0.0521	0.0567	0.7787	0.6882
11	0.3075	0.2582	0.1290	0.0720
ADIPOR2				
00	0.5079	0.5470	0.2782	0.0926
10	0.1011	0.0761	0.2226	0.0585
11	0.3836	0.3614	0.5249	0.1326

The '0' and '1' used for haplotype notation stand for 'major allele' and 'minor allele', respectively

NDM Non-diabetic subjects; *T2DM* type 2 diabetic subjects

caused by multiple testing; threshold of significance, $p=0.001$), no association was found between HOMA-IR and SNP15 in *ADIPOR2*. Moreover, when SNP15 in *ADIPOR2* was further genotyped in 384 additional type 2 diabetic subjects and 384 additional non-diabetic subjects (second panel) to avoid type 2 errors, no significant differences were observed in the HOMA results when compared according to SNP15 genotype (11/12/22: 1.66±0.09/1.62±0.07/1.67±0.13, $p=0.91$). There were no differences in clinical parameters, such as sex, age, BMI, HbA_1c and fasting glucose, between the genotypes of any of the SNPs investigated in the present study (data not shown). We then performed a haplotype analysis, which may be a more sensitive method for detecting associations than the assessment of individual SNPs. First, the haplotype blocks in *ADIPOR1* and *ADIPOR2* were determined. The tagged SNPs that represented more than 90% of the haplotypes in each block were then selected, and the difference in the frequency of each haplotype between the type 2 diabetic subjects and the non-diabetic subjects was analysed. As shown in Table 3, none of the haplotypes in *ADIPOR1* or *ADIPOR2* were associated with type 2 diabetes.

Discussion

After constructing a dense map of SNPs in *ADIPOR1* and *ADIPOR2* and performing haplotype analysis, no evidence of a major role for *ADIPOR1* or *ADIPOR2* in susceptibility to type 2 diabetes or insulin resistance was found in a Japanese population. Our results may reflect a type 2 error (false-negative result), but this is unlikely. First, SNP densities of one SNP every 1.2 kb in *ADIPOR1* and one SNP every 3.9 kb in *ADIPOR2* were used for the association study. The distance between each SNP was short, and the LD between them was fully analysed. We estimated that more than 90% of the haplotypes in *ADIPOR1* and *ADIPOR2* were covered. Second, the sample size used in the present study had an 80% power to detect the effect of a polymorphism, conferring an odds ratio of 2.0 at a significance level of 5% (assuming an allele frequency of 40% in the control population). However, it cannot be excluded that SNPs in *ADIPOR1* and/or *ADIPOR2* had a minor effect on susceptibility to type 2 diabetes.

Consistent with our results for *ADPOR1*, an American study recently reported that SNPs in *ADIPOR1* were not associated with type 2 diabetes in Caucasians or African Americans [20]. However, they reported that the level of expression of *ADIPOR1* in lymphocytes from type 2 diabetic subjects was reduced compared with that in lymphocytes from non-diabetic subjects, implicating *ADIPOR1* in the pathogenesis of type 2 diabetes. Further analysis is needed to clarify the role played by *ADIPOR2* in susceptibility to type 2 diabetes in different ethnic groups.

In summary, the genetic variations in *ADIPOR1* or *ADIPOR2* investigated in the present study were not associated with insulin resistance or type 2 diabetes. However, further studies using denser SNPs and larger samples may be required to conclusively determine that genetic variations in *ADIPOR1* or *ADIPOR2* are not major genetic determinants of the development of type 2 diabetes or insulin resistance.

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Adiponectin and Adiponectin Receptors

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Metabolic syndrome is thought to result from obesity and obesity-linked insulin resistance. Obesity in adulthood is characterized by adipocyte hypertrophy. Adipose tissue participates in the regulation of energy homeostasis as an important endocrine organ that secretes a number of biologically active "adipokines."

Heterozygous peroxisome proliferator-activated receptor-γ knockout mice were protected from high-fat diet induced obesity, adipocyte hypertrophy, and insulin resistance. Systematic gene profiling analysis of these mice revealed that adiponectin/Acrp30 was overexpressed. Functional analyses including generation of adiponectin transgenic or knockout mice have revealed that adiponectin serves as an insulin-sensitizing adipokine. In fact, obesity-linked down-regulation of adiponectin was a mechanism whereby obesity could cause insulin resistance and diabetes.

Recently, we have cloned adiponectin receptors in the skeletal muscle (AdipoR1) and liver (AdipoR2), which appear to comprise a novel cell-surface receptor family. We showed that AdipoR1 and AdipoR2 serve as receptors for globular and full-length adiponectin and mediate increased AMP-activated protein kinase, peroxisome proliferator-activated receptor-α ligand activities, and glucose uptake and fatty-acid oxidation by adiponectin. Obesity decreased expression levels of AdipoR1/R2, thereby reducing adiponectin sensitivity, which finally leads to insulin resistance, the so-called "vicious cycle." Most recently, we showed that osmotin, which is a ligand for the yeast homolog of AdipoR (PHO36), activated AMPK via AdipoR in C2C12 myocytes. This may facilitate efficient development of adiponectin receptor agonists.

Adiponectin receptor agonists and adiponectin sensitizers should serve as versatile treatment strategies for obesity-linked diseases such as diabetes and metabolic syndrome. (*Endocrine Reviews* 26: 439–451, 2005)

Abbreviations: ACC, Acetyl-coenzyme-A carboxylase; AMPK, AMP-activated protein kinase; apoE, apolipoprotein E; EGF, epidermal growth factor; gAd, globular adiponectin; HF, high-fat; HMW, high molecular weight; PI, phosphatidylinositol; PPAR, peroxisome proliferator-activated receptor; PR, pathogenesis-related; siRNA, small interfering RNA; SNP, single nucleotide polymorphism; TG, triglyceride.

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I. Introduction

HIGH-FAT (HF) diet-induced insulin resistance associated with obesity is a major risk factor for diabetes and cardiovascular diseases, the prevalence of which is increasing sharply (1, 2). However, the molecular basis for this association remains to be elucidated. The adipose tissue itself serves as the site of triglyceride (TG) storage and free fatty acid/glycerol release in response to changing energy demands (1). Adipose tissue also participates in the regulation of energy homeostasis as an important endocrine organ that secretes a number of biologically active adipokines such as free fatty acid (3), adipsin (4), leptin (5), plasminogen activator inhibitor-1 (6), resistin (7), and TNF-α (8). Adiponectin is one such adipokine that has recently attracted much attention. In this review, we will describe recent progress made in adiponectin research with particular emphasis on the role of adiponectin in the regulation of insulin sensitivity and the development of insulin resistance. Other aspects of adiponectin research have been reviewed elsewhere (9–14).

II. Identification and Molecular Structure

A. *Identification*

Adiponectin was originally identified independently by four groups using different approaches. Mouse cDNAs for

adiponectin, termed Acrp30 (15) and AdipoQ (16), were cloned by differential display before and after differentiation of mouse 3T3-L1 and 3T3-F442A cells. Human adiponectin cDNA was isolated by large-scale random sequencing of a 3′-directed human adipose tissue cDNA library (17). Human adiponectin was also purified from plasma as a gelatin binding protein, GBP28 (18).

B. Molecular structure and multimeric form of adiponectin

Adiponectin structurally belongs to the complement 1q family (19–21) (Fig. 1) and is known to form a characteristic homomultimer (22) (Fig. 2). It has been demonstrated that simple SDS-PAGE under nonreducing and non-heat-denaturing conditions clearly separates multimeric species of adiponectin (23) (Fig. 2). Adiponectin in human or mouse serum and adiponectin expressed in NIH-3T3 cells or *Escherichia coli* forms a wide range of multimers from trimers and hexamers to high molecular weight (HMW) multimers (23) such as dodecamers and 18 mers, as demonstrated by ourselves and other groups (22, 24, 25) (Fig. 2).

Adiponectin can exist as full-length or a smaller, globular fragment; however, almost all adiponectin appears to exist as full-length adiponectin in plasma. Lodish's group reported that a small amount of globular adiponectin was detected in human plasma (26) (Fig. 1). It has been proposed that the globular fragment is generated by proteolytic cleavage, and recently it has been shown that the cleavage of adiponectin by leukocyte elastase secreted from activated monocytes and/or neutrophils could be responsible for the generation of the globular fragment of adiponectin (27). However, the pathophysiological importance of adiponectin cleavage by leukocyte elastase *in vivo* remains to be determined.

Oligomer formation of adiponectin depends on disulfide bond formation mediated by Cys-39 (28). Interestingly, a mutant adiponectin with a substitution of Cys by Ser at codon 39, which formed a trimer and readily underwent proteolytic cleavage, showed much more potent bioactivity, such as reduction of glucose output from primary hepatocytes, than wild-type adiponectin with a HMW.

Hydroxylation and glycosylation of the four lysines in the collagenous domain of adiponectin have been shown to play important roles in enhancing the ability of subphysiological concentrations of insulin to inhibit gluconeogenesis in hepatocytes (29). Adiponectin was reported to be an α2,8-linked disialic acid-containing glycoprotein, although the biological functions of the disialic acid epitope of adiponectin remain to be elucidated (30).

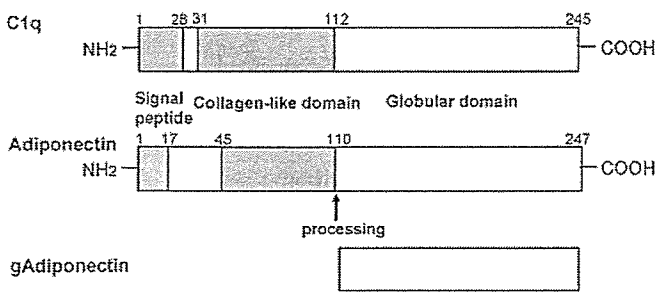

FIG. 1. Structure and domains of adiponectin. Adiponectin, also known as Acrp30, AdipoQ, and GBP28, was originally identified independently by four groups using different approaches (15–18). Adiponectin is composed of an N-terminal collagen-like sequence and a C-terminal globular region. A small amount of a processed globular form was reported to be present in human plasma (26).

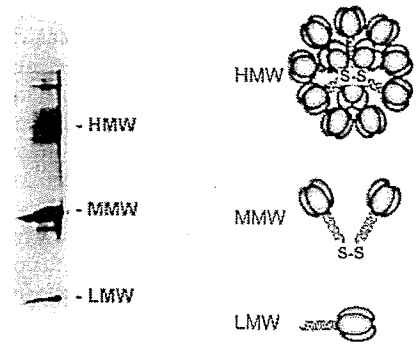

FIG. 2. Multimer formation of adiponectin. Human serum was subjected to SDS-PAGE under nonreducing, non-heat-denaturing conditions, and multimer forms of adiponectin were detected using antiadiponectin antibody (23). MMW, Medium molecular weight; LMW, low molecular weight; S–S, disulfide bridge.

III. Adiponectin and Insulin Resistance

A. Low plasma adiponectin levels and insulin resistance

Spiegelman's group reported that adiponectin expression is exclusive to adipose tissue and that the mRNA expression of adiponectin was reduced in obese diabetic murine model db/db mice (16). Plasma levels of adiponectin have also been reported to be significantly reduced in obese/diabetic mice and humans (16, 31, 32). Moreover, plasma adiponectin levels have been shown to be decreased in patients with cardiovascular diseases (33, 34), hypertension (35), or metabolic syndrome (36). Thus, reductions in plasma adiponectin levels are commonly observed in a variety of states frequently associated with insulin resistance. However, whether this apparent parallelism between low plasma adiponectin levels and insulin resistance represents a cause and effect relationship was not known.

B. Insulin-sensitizing effects of adiponectin

The insulin-sensitizing effect of adiponectin was first identified by three independent groups in 2001 (26, 31, 37). We previously generated heterozygous PPAR (peroxisome proliferator-activated receptor) γ knockout mice that remained insulin-sensitive on a HF diet (38). In an attempt to identify insulin-sensitizing molecules secreted from white adipose tissue of heterozygous PPARγ knockout mice, oligonucleotide microarray analysis was carried out using white adipose tissue (39). Adiponectin as well as leptin expression was up-regulated. Leptin was previously shown to be a major insulin-sensitizing adipokine (40).

To verify the direct insulin-sensitizing effect of adiponectin *in vivo*, an insulin-resistant lipoatrophic diabetic mouse model that displays both adiponectin and leptin deficiency was employed (41). Replenishment of a physiological dose of recombinant adiponectin to the lipoatrophic diabetic mice significantly ameliorated insulin resistance (31). Moreover,

insulin resistance in lipoatrophic mice was completely reversed by the combination of physiological doses of adiponectin and leptin, but only partially by either adiponectin or leptin alone (31). These data clearly indicate that adiponectin has a direct insulin-sensitizing action. These data also suggest that leptin and adiponectin may be the two major insulin-sensitizing hormones secreted from adipose tissue.

We also studied whether adiponectin can improve insulin resistance and diabetes in murine models of type 2 diabetes, characterized by obesity, insulin resistance, and hyperglycemia. Serum adiponectin levels were decreased in KKAy mice on a HF diet compared with those under a high-carbohydrate diet (31) (Fig. 3). Lower serum adiponectin levels in KKAy mice on the HF diet were partially restored by replenishment of recombinant adiponectin. Importantly, replenishment of adiponectin significantly ameliorated HF diet-induced insulin resistance and hypertriglyceridemia (31) (Fig. 3). These data suggest that the insulin resistance associated with HF diets and obesity is caused at least in part by the decreases in adiponectin linked to those circumstances. The data suggest that the fat-derived hormone adiponectin is decreased in obesity and deficient in lipoatrophy, and that reduction in adiponectin plays causal roles in the development of insulin resistance in these models.

Scherer's group has reported that an acute increase in circulating adiponectin levels triggers a transient decrease in basal glucose levels by inhibiting both the expression of hepatic gluconeogenic enzymes and the rate of endogenous glucose production in both wild-type mice and a type 2 diabetes mouse model (37, 42), consistent with the proposal that adiponectin sensitizes the body to insulin. Lodish's group reported that a proteolytic cleavage product of adiponectin increases fatty-acid oxidation in muscle and causes a decrease in plasma glucose and weight loss in mice (26).

These data raise the possibility that the replenishment of adiponectin may provide a novel treatment modality for insulin resistance and type 2 diabetes.

IV. Mouse Models

The chronic effects of adiponectin on insulin resistance *in vivo* were investigated by generating globular adiponectin transgenic mice (43, 44) or adiponectin-deficient mice (45–47). Globular adiponectin transgenic mice were generated and crossed with ob/ob mice (45). Globular adiponectin transgenic ob/ob mice showed partial amelioration of insulin resistance and diabetes, but not of obesity (43). These data suggested that chronic elevation of globular adiponectin has a direct insulin-sensitizing effect independent of white adipose tissue mass.

Scherer's group reported that transgenic mice with a deletion in the collagenous domain of adiponectin displayed 3-fold elevated levels of circulating adiponectin, raised lipid clearance and lipoprotein lipase activity, and improved insulin-mediated suppression of endogenous glucose production, thereby improving insulin sensitivity (44). In rats, sustained peripheral expression of adiponectin by the transgene also offset the development of diet-induced obesity (48).

Globular adiponectin transgenic mice were also crossed with apolipoprotein E (apoE)-deficient mice to study whether globular adiponectin can inhibit atherosclerosis *in vivo* (43). apoE-Deficient mice are hypercholesterolemic and spontaneously develop severe atherosclerosis. We compared the extent of atherosclerotic lesions of globular adiponectin transgenic apoE-deficient mice to that in control apoE-deficient mice. Although serum parameters such as total cholesterol, TG, glucose, and insulin were not altered, the *en face* Sudan IV-positive lesion areas of the arch and the descending aorta were significantly smaller in globular adiponectin transgenic apoE-deficient mice than in control apoE-deficient littermates (43). Similar results were obtained by using adenoviral-mediated overexpression of adiponectin in apoE knockout mice (49). Thus, overexpression of adiponectin resulted in marked reduction of atherosclerotic lesion formation. Together with the observations that adiponectin can ameliorate diabetes and hyperlipidemia, adiponectin can re-

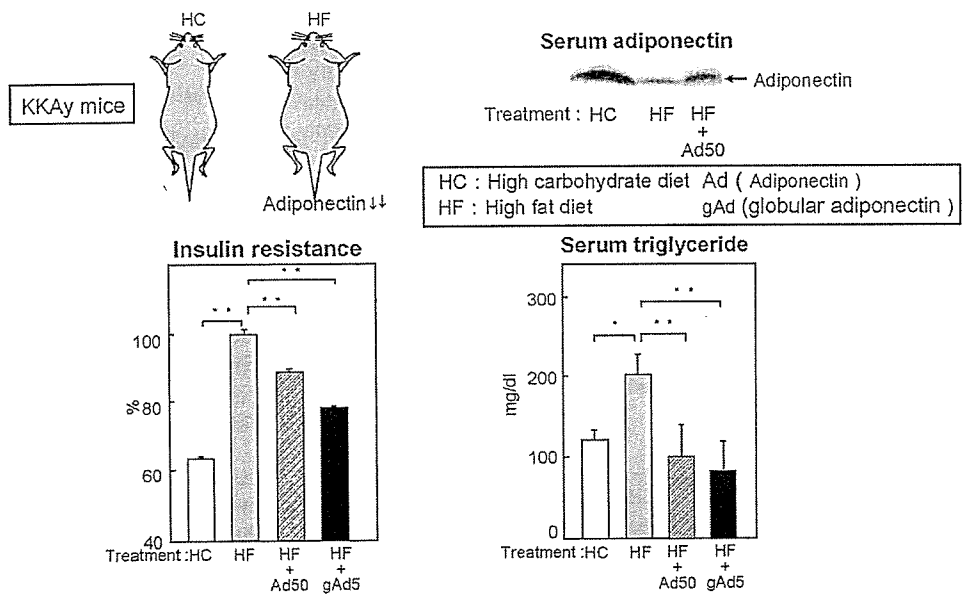

FIG. 3. Replenishment of adiponectin reversed insulin resistance and metabolic syndrome in a murine model of type 2 diabetes. Serum adiponectin levels were decreased in mice on a HF diet compared with those in mice on a high-carbohydrate (HC) diet. Lower serum adiponectin levels in mice on the HF diet were partially restored compared with those in mice on the HC diet by replenishment of recombinant adiponectin, which significantly ameliorated HF diet-induced insulin resistance. *, $P < 0.05$; **, $P < 0.01$, between the two groups indicated.

duce atherosclerosis both via direct effects on vascular wall and via reduction in risk factors.

To determine the physiological role of adiponectin, we and others have generated adiponectin knockout mice and reported that adiponectin-deficient mice exhibited characteristics of the metabolic syndrome such as insulin resistance, glucose intolerance, hyperlipidemia, and hypertension (35, 45, 46).

We and others also studied the role of adiponectin in vascular wall using adiponectin knockout mice (45, 50). We placed a cuff around the femoral artery to induce inflammation of the adventitia and subsequent neointimal formation 2 wk after cuff placement. Intimal thickness was significantly greater (2-fold) in adiponectin knockout mice than in the wild-type mice. Thus, adiponectin plays a protective role against neointimal formation in response to injury (45, 50).

V. Mechanism of Action of Adiponectin

A. *Insulin-sensitizing actions*

1. Adiponectin reduces tissue TG content and up-regulates insulin signaling. Interestingly, in skeletal muscle, adiponectin increased expression of molecules involved in fatty-acid transport such as CD36, in combustion of fatty-acid such as acyl-coenzyme A oxidase, and in energy dissipation such as uncoupling protein 2. These changes led to decreased tissue TG content in skeletal muscle (31).

Increased tissue TG content has been reported to interfere with insulin-stimulated phosphatidylinositol (PI) 3-kinase activation and subsequent glucose transporter 4 translocation and glucose uptake, leading to insulin resistance (3). Thus, decreased tissue TG content in muscle may contribute to improved insulin signal transduction. This was demonstrated in skeletal muscle of lipoatrophic mice treated with adiponectin, in which increases in insulin-induced tyrosine phosphorylation of insulin receptor and insulin receptor substrate-1 and insulin-stimulated phosphorylation of Akt were seen (31).

2. Adiponectin activates PPARα. Based on the data that treatment of lipoatrophic or obese diabetic mice with adiponectin or overexpression of adiponectin in ob/ob mice resulted in increased expression levels of PPARα target genes such as CD36, acyl-coenzyme A oxidase, and uncoupling protein 2, we hypothesized that adiponectin could activate PPARα (31) (Fig. 4).

Consistent with this hypothesis, adiponectin indeed increased the expression levels of PPARα *in vivo* (31). These data suggested that adiponectin increased fatty-acid combustion and energy consumption, presumably via PPARα activation at least in part, which led to decreased TG content in the liver and skeletal muscle and thus coordinately increased *in vivo* insulin sensitivity.

Endogenous PPARα ligand activities were measured *in vitro* to further clarify the mechanisms by which adiponectin activated PPARα (31, 43). Interestingly, the treatment of C2C12 myocytes with adiponectin for 6 h significantly increased PPARα ligand activities (43) and at the same time fatty-acid oxidation *in vitro*.

3. Adiponectin activates AMP kinase. We next examined the effects of treatment of adiponectin for a shorter time period

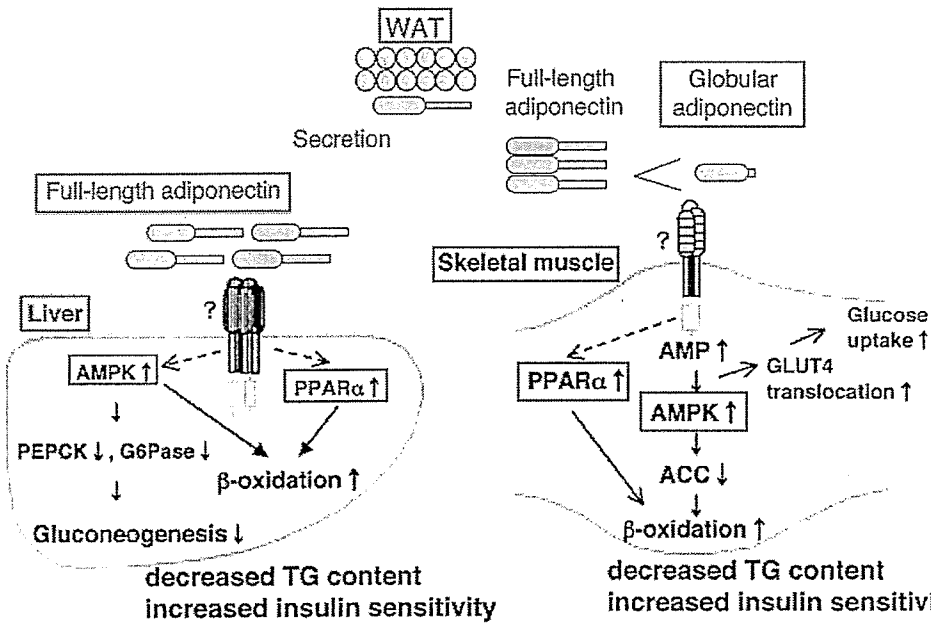

FIG. 4. Adiponectin can activate AMPK and PPARα in the liver and skeletal muscle. In skeletal muscle, both globular and full-length adiponectin activate AMPK, thereby stimulating phosphorylation of ACC, fatty-acid oxidation, and glucose uptake. Adiponectin activates PPARα, thereby also stimulating fatty-acid oxidation and decreasing tissue TG content in muscle. In the liver, only full-length adiponectin activates AMPK, thereby reducing molecules involved in gluconeogenesis and increasing phosphorylation of ACC and fatty-acid oxidation. Adiponectin activates PPARα, thereby stimulating fatty-acid oxidation and decreasing tissue TG content in the liver. These alterations all increase insulin sensitivity *in vivo* (101). WAT, White adipose tissue; PEPCK, phosphoenolpyruvate carboxykinase; G6Pase, glucose-6-phosphatase G6Pase.

(51). Treatment of C2C12 myocytes with adiponectin for 1 h stimulated fatty-acid oxidation. Although actinomycin D had no effect on the increase in fatty-acid oxidation stimulated by adiponectin for 1 h, it suppressed fatty-acid oxidation stimulated by the PPARα agonist Wy-14,643. Moreover, treatment of C2C12 myocytes for 1 h stimulated glucose uptake. We hypothesized that adiponectin may stimulate β-oxidation and glucose uptake via AMP-activated protein kinase (AMPK) during a period shorter than 6 h (51).

Globular adiponectin and full-length adiponectin stimulated phosphorylation and activation of AMPK in skeletal muscle, whereas only full-length adiponectin did so in the liver (51). In parallel with its activation of AMPK, adiponectin stimulated phosphorylation of acetyl coenzyme-A carboxylase (ACC), fatty-acid combustion, glucose uptake, and lactate production in myocytes, and also stimulated phosphorylation of ACC and caused a reduction in molecules involved in gluconeogenesis in the liver, which can account for the acute glucose-lowering effects of adiponectin *in vivo* (51). Blocking AMPK activation by use of a dominant negative mutant inhibited each of these effects, indicating that stimulation of glucose utilization and fatty-acid combustion by adiponectin occurs through activation of AMPK. Our data may provide a novel paradigm that an adipocyte-derived hormone activates AMPK, thereby directly regulating glucose metabolism and insulin sensitivity *in vitro* and *in vivo* (51) (Fig. 4).

The group of Lodish and Ruderman also showed that the adiponectin/ACRP30 globular domain enhanced muscle fat oxidation and glucose transport via AMPK activation and ACC inhibition (52). More recently, AMPK was reported to be involved in glucose uptake stimulated by the globular domain of adiponectin in primary rat adipocytes (53). Because leptin has also been shown to stimulate AMPK in skeletal muscle (54), activation of AMPK may be a common mechanism by which insulin-sensitizing adipokines such as adiponectin and leptin increase insulin sensitivity.

Scherer's group also reported that in adiponectin transgenic mice, reduced expression of gluconeogenic enzymes such as phosphoenolpyruvate carboxykinase and glucose-6-phosphatase was associated with elevated phosphorylation of AMPK in liver (44). The same group reported that adiponectin is found as two forms in serum, as a lower molecular weight trimer-dimer and a HMW complex (28). Female subjects display significantly higher levels of the HMW complex in serum than do male subjects (23, 28, 55–57). Levels of the HMW complex appeared to be negatively regulated by insulin. In accordance with this, the amount of HMW adiponectin complex, but not the total amount of adiponectin, was recently reported to be correlated with a thiazolidinedione-mediated improvement in insulin sensitivity (55).

B. Antiatherosclerotic actions

Adiponectin has been reported to have direct antiatherosclerotic effects (58–67). Adiponectin was demonstrated to strongly inhibit the expression of adhesion molecules, including intracellular adhesion molecule-1, vascular cellular adhesion molecule-1, and E-selectin (Fig. 5). Adiponectin was also shown to inhibit TNF-α-induced nuclear factor-κB activation through the inhibition of IκB phosphorylation (61). Suppression of nuclear factor-κB by adiponectin might be a major molecular mechanism for the inhibition of monocyte adhesion to endothelial cells (62). Adiponectin also inhibits the expression of the scavenger receptor class A-1 of macrophages, resulting in markedly decreased uptake of oxidized low-density lipoprotein by macrophages and inhibition of foam cell formation (63). In addition, in cultured smooth muscle cells, adiponectin attenuated DNA synthesis

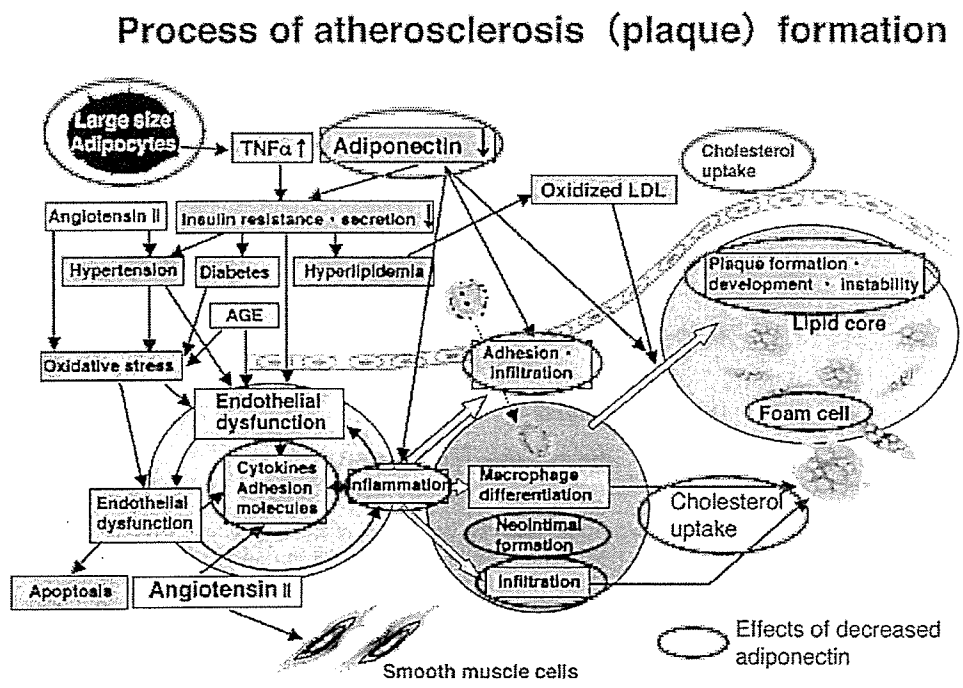

FIG. 5. Suppression of atherosclerosis by adiponectin. Adiponectin inhibits atherosclerosis and plaque formation at least via suppression of two processes: 1) suppression of neointimal formation by inhibiting the expressions of inflammatory cytokines and adhesion molecules; and 2) suppression of uptake of cholesterol by inhibiting the expression of scavenger receptors. LDL, Low-density lipoprotein.

induced by growth factors including platelet-derived growth factor, heparin-binding epidermal growth factor (EGF)-like growth factor, basic fibroblast growth factor, and EGF, as well as cell proliferation and migration induced by heparin-binding EGF-like growth factor (64). This inhibition was shown to be attributable to the inhibition of signal transduction through ERK. More recently, selective suppression of endothelial cell apoptosis via AMPK activation by the HMW form of adiponectin has been reported (65).

VI. Alterations in Adiponectin Gene Are Associated with Human Diabetes

Independent of these functional analyses carried out *in vitro* and in animal models, data from human genetic studies on adiponectin also support the role of adiponectin as a determinant of susceptibility to insulin resistance and type 2 diabetes. By use of affected sib-pair analysis, complete genome mapping of type 2 diabetes genes in Japanese was performed (68). The genome scans revealed at least nine chromosomal regions linked to type 2 diabetes in Japanese people. Among these, three chromosomal regions (3q, 15q, and 20q) are the same regions as previously reported in other ethnic groups. Among these three chromosomal regions, interestingly, the 3q27 chromosomal region contains the adiponectin gene.

We screened for the adiponectin gene and identified 10 relatively common single nucleotide polymorphisms (SNPs) in the Japanese population (Fig. 6). One such SNP, SNP 276 in intron 2 (G *vs.* T), showed interesting phenotypes with respect to plasma adiponectin levels, insulin resistance, and susceptibility to type 2 diabetes (69) (Fig. 7). Subjects with the G/G genotype had lower plasma adiponectin levels than those with the T/T genotype. Subjects with the G/G genotype at position 276 had a higher insulin resistance index than those with T/T. Importantly, subjects with the G/G genotype at position 276 were at increased risk for type 2 diabetes. The odds ratio was slightly greater than 2 (69) (Fig. 8). Similar associations for the adiponectin gene with susceptibility to type 2 diabetes have also been reported in other ethnic groups (70–72). In German and American Caucasians, the SNP 276, either independently or as a haplotype together with SNP 45 in exon 2, was shown to be associated with obesity and insulin resistance (71, 72). In French Caucasians, two SNPs in the promoter region of the adiponectin gene, SNP-11377 and SNP-11391, were significantly associated with hypoadiponectinemia and type 2 diabetes (70). Taken together, these data strongly support the hypothesis that adiponectin plays a pivotal role in the pathogenesis of type 2 diabetes.

Several cross-sectional studies have reported that adiponectin levels were decreased in subjects with type 2 diabetes and are inversely correlated with insulin resistance. However, no studies had investigated whether adiponectin protects subjects from diabetes or the extent of risk of developing diabetes in subjects with hypoadiponectinemia. Recently, matched case-control studies in subjects recruited from a large cohort have examined the protective effect of adiponectin against diabetes. One study was performed in severely obese Pima Indian subjects, who have the highest known prevalence of obesity and type 2 diabetes in the world, to assess the role of adiponectin independent of the effects of obesity (73). Subjects with high concentrations of adiponectin were 40% less likely to develop type 2 diabetes than those with low concentrations after adjustment for body mass index (BMI), indicating that adiponectin could be used as a predictor of future development of type 2 diabetes in addition to the established risk parameters, such as BMI.

In addition to the relatively common SNPs, eight mutations in the human adiponectin gene have been reported (69, 74, 75), some of which were significantly related to diabetes and hypoadiponectinemia (23, 75). Among human adiponectin mutations, Arg112Cys and Ile164Thr mutants did not assemble into trimers, which caused impaired secretion from the cell (23). These mutants are clinically associated with hypoadiponectinemia. The Gly84Arg and Gly90Ser mutants were able to assemble into trimers and hexamers but were unable to form HMW multimers (the HMW multimers are thought to be larger than heximers), which are clinically associated with diabetes. These data raised the possibility that HMW multimers have more potent insulin-sensitizing effects than trimers and hexamers (23).

These data suggest that impaired multimerization of adiponectin may be among the causes of a diabetic phenotype or hypoadiponectinemia in subjects having these mutations. Thus, not only the total concentrations but also the multimer distribution should always be considered when interpreting plasma adiponectin levels in health as well as various disease states (23–25).

VII. Cloning of Adiponectin Receptors AdipoR1 and AdipoR2

We believe that cloning of the adiponectin receptor should facilitate studies on the regulation of glucose and lipid me-

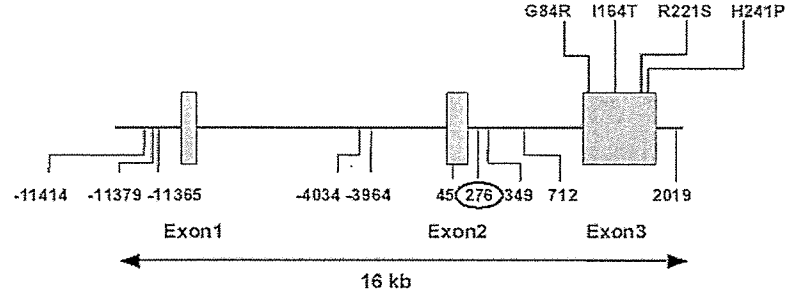

FIG. 6. Schema of genomic structure and polymorphic variants of the adiponectin gene. Exon-intron organization of the gene is indicated by *closed boxes*. *Arrows* show the positions of the polymorphic variants identified. *Numbers* indicate locations relative to the A of the ATG of the initiator Met of the adiponectin gene. Rare mutations with amino acid substitutions are also described (23, 70, 75).

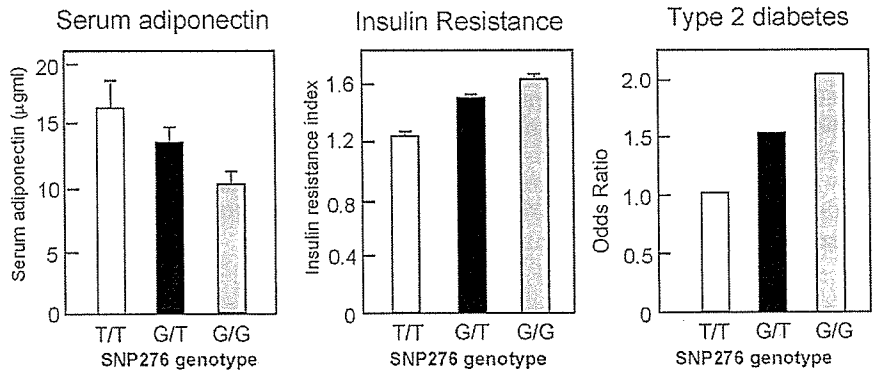

FIG. 7. Effects of SNP 276 in intron 2 of the adiponectin gene on serum adiponectin, insulin resistance, and susceptibility to type 2 diabetes. The effects of SNP 276 in intron 2 on plasma adiponectin levels, insulin resistance, and susceptibility to type 2 diabetes were studied. On the *left*, subjects with the G/G genotype had lower plasma adiponectin levels than those with the T/T genotype. In the *middle*, subjects with the G/G genotype at position 276 had a higher insulin resistance index than those with T/T. Importantly, on the *right*, subjects with the G/G genotype at position 276 were at increased risk for type 2 diabetes. The odds ratio was slightly greater than 2.

tabolism, the molecular causes of diabetes and atherosclerosis, and the development of antidiabetic and antiatherosclerotic drugs. We isolated cDNA for adiponectin receptors (AdipoR) that mediate the antidiabetic effects from human skeletal muscle cDNA library by screening for globular adiponectin binding (76).

The cDNA analyzed encoded a protein designated human AdipoR1 (Fig. 8) (76). This protein is conserved from yeast to man (especially in the seven transmembrane domains). Interestingly, this yeast homolog YOL002c plays a key role in metabolic pathways that regulate lipid metabolism such as fatty-acid oxidation (77).

Because there may be two distinct adiponectin receptors, we searched for a homologous gene in the human and mouse databases. We found only one gene that was significantly homologous (67% identity in amino acids) to AdipoR1, which was termed AdipoR2 (Fig. 8) (76). AdipoR1 was ubiquitously expressed and most abundantly expressed in skeletal muscle, whereas AdipoR2 was most abundantly expressed in mouse liver. It was reported that adiponectin receptors were expressed in pancreatic β-cells, and that fatty acids may regulate their expression levels (78). GH is reported to be a positive regulator of AdipoR2 in 3T3-L1 adipocytes (79).

AdipoR1 and AdipoR2 appeared to be integral membrane proteins; the N terminus was internal, and the C terminus was external, which is opposite to the topology of all other reported G protein-coupled receptors (Fig. 8) (76). AdipoR1 and AdipoR2 may form both homo- and heteromultimers. Scatchard plot analysis revealed that AdipoR1 is a receptor for globular adiponectin, whereas AdipoR2 is a receptor for full-length adiponectin (76). Suppression of AdipoR1 with small interfering RNA (siRNA) reduced the increase in fatty-acid oxidation by globular adiponectin. Suppression of AdipoR2 with siRNA reduced the increase in fatty-acid oxidation by full-length adiponectin (Fig. 9) (76).

Thus, we have isolated cDNA-encoding adiponectin receptors (AdipoR1 and R2). Expression of AdipoR1/R2 or suppression of AdipoR1/R2 supports our conclusion that AdipoR1 and R2 serve as receptors for globular and full-length adiponectin and mediate increased AMPK, PPARα ligand activities and fatty-acid oxidation and glucose uptake by adiponectin (Fig. 9) (57).

Lodish's group reported that T-cadherin was capable of binding adiponectin in C2C12 myoblasts, but not in the liver or hepatocytes (80).

VIII. Regulation of Adiponectin Receptors

A. Regulation of expression levels of AdipoR1 and AdipoR2

We first examined whether the expressions of AdipoR1 and/or AdipoR2 were regulated under physiological and/or pathophysiological states (81). The levels of AdipoR1 and AdipoR2 mRNA expression in the liver and skeletal muscle increased after fasting, and refeeding rapidly restored these to levels equal to the original fed state. AdipoR1 and AdipoR2 mRNA increased significantly in skeletal muscle of

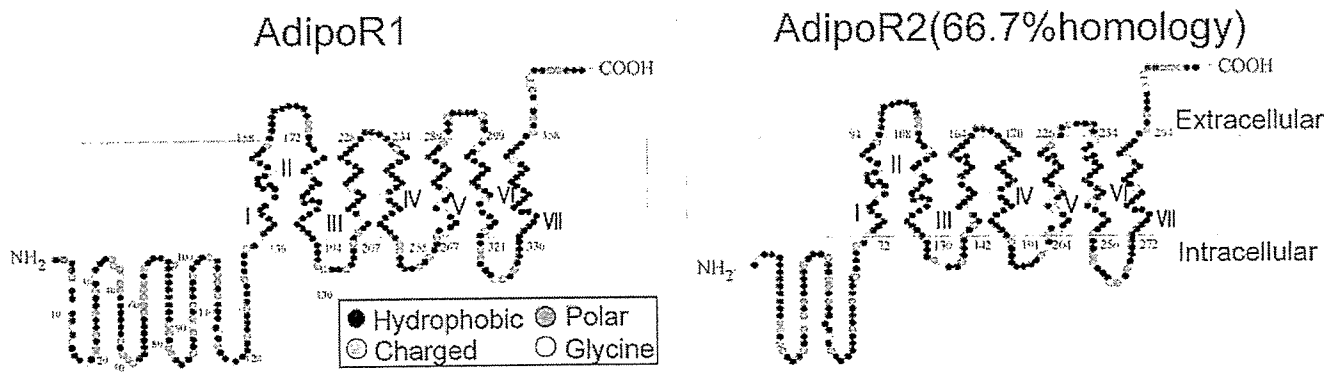

FIG. 8. Proposed structure of adiponectin receptors and their expression in various tissues. cDNA encoding adiponectin receptors (AdipoR1 and R2) were isolated. AdipoR1 was ubiquitously expressed and most abundantly expressed in skeletal muscle, whereas AdipoR2 was most abundantly expressed in mouse liver (76).

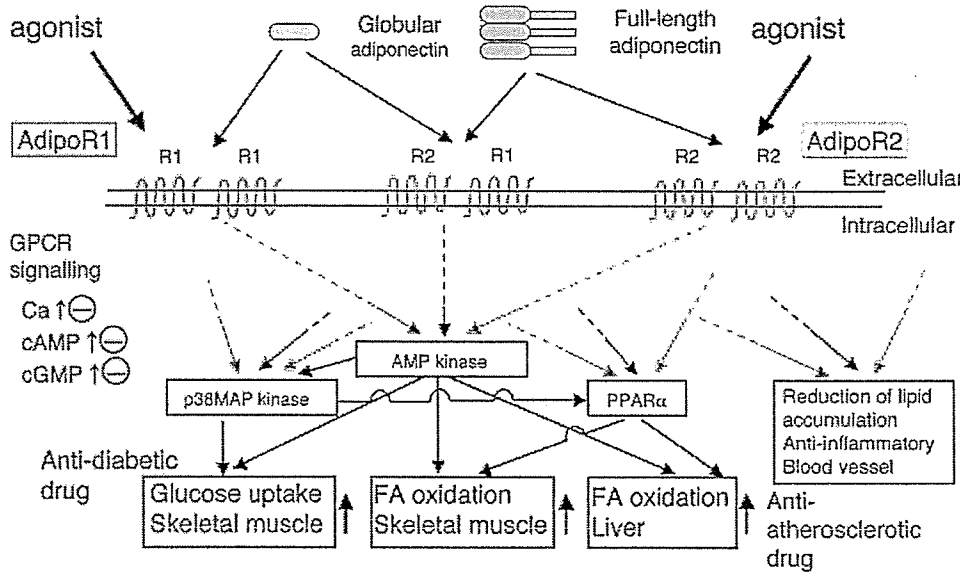

FIG. 9. Molecular mechanisms of adiponectin action. cDNA encoding adiponectin receptors (AdipoR1 and R2) was isolated. Expression of AdipoR1/R2 or suppression of AdipoR1/R2 supports the conclusion that AdipoR1 and R2 serve as receptors for globular and full-length adiponectin and mediate increased AMPK, PPARα ligand activities, and fatty-acid oxidation and glucose uptake by adiponectin. Molecular cloning of AdipoR1 and R2 should facilitate the designing of novel antidiabetic and antiatherogenic drugs with AdipoR1 and R2 as molecular targets (76, 101). FA, Fatty acid; GPCR, G protein-coupled receptor.

mice rendered hypoinsulinemic/hyperglycemic with streptozotocin, and both AdipoR1 and AdipoR2 mRNA were almost completely restored by insulin treatment. These observations suggested that insulin may negatively regulate AdipoR1/R2 mRNA levels (81). The PI3-kinase inhibitor LY 294002 and constitutively active form of Foxo (Forkhead box, class O) 1 revealed that insulin repressed AdipoR1/R2 mRNA expressions via activation of PI3-kinase and inactivation of Foxo1 (81).

The expressions of both AdipoR1 and AdipoR2 were significantly decreased in muscle and adipose tissue of insulin-resistant ob/ob mice, which exhibited hyperglycemia and hyperinsulinemia, as compared with control mice (81) (Fig. 10). Scatchard plot analysis revealed that both high-affinity and low-affinity binding sites for globular adiponectin (gAd) and adiponectin binding in skeletal muscles of ob/ob mice were reduced as compared with those of wild-type mice, findings that are consistent with the fact that the numbers of both AdipoR1 and AdipoR2 were reduced. Moreover, adiponectin-induced activation of AMPK was impaired in skeletal muscle of ob/ob mice. These data suggest that adiponectin resistance was observed in ob/ob mice, which exhibited decreased expression levels of AdipoR1 and AdipoR2 (81) (Fig. 10).

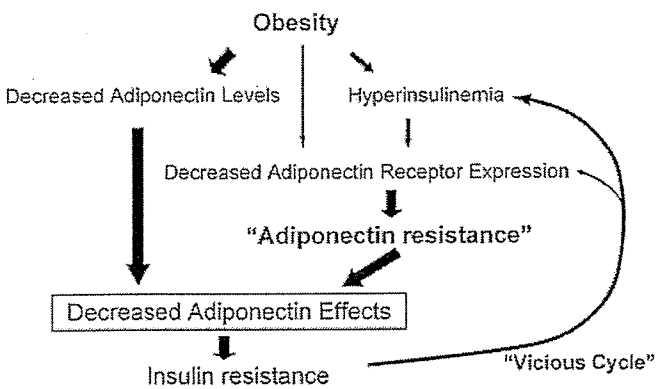

FIG. 10. Obesity, adiponectin resistance, and insulin resistance. Plasma adiponectin levels were decreased in obesity, which may play causal roles in the development of insulin resistance. The expression levels of AdipoR1/R2 were also decreased in obesity. Obesity decreased expression levels of AdipoR1/R2, thereby reducing adiponectin sensitivity, which finally led to insulin resistance, the so-called "vicious cycle". These data also suggest that not only agonism of AdipoR1/R2 but also strategies to increase AdipoR1/R2 may be a logical approach to provide a novel treatment modality for insulin resistance and type 2 diabetes (81).

We and others have previously shown that plasma adiponectin levels were decreased in obesity. This reduction may play a causal role in the development of insulin resistance. In the same study, we have also shown that obesity decreased the expression levels of AdipoR1/R2, thereby reducing adiponectin sensitivity, which finally leads to insulin resistance, the so-called "vicious cycle" (61) (Fig. 10).

A correlation has been reported between adiponectin receptor gene expression and insulin sensitivity in nondiabetic Mexican Americans with or without a family history of type 2 diabetes (82). Adiponectin receptor expression in skeletal muscle of type 2 diabetic patients was also reported to be decreased (83).

Our data suggest that not only agonism of AdipoR1/R2 but also strategies to increase AdipoR1/R2 may be a logical approach with which to provide a novel treatment modality for insulin resistance and type 2 diabetes.

IX. Adiponectin Hypothesis

Based upon the significant body of evidence discussed in this review, obtained from our and other laboratories, we propose the following adiponectin hypothesis (Fig. 11). Reduced adiponectin levels were caused by interactions of genetic factors such as SNPs in the adiponectin gene itself and environmental factors causing obesity such as a HF diet. Reduced adiponectin actions also resulted from down-regulation of adiponectin receptors linked to obesity. These reductions of adiponectin actions may play a crucial causal role in the development of insulin resistance, type 2 diabetes, metabolic syndrome, and atherosclerosis (Fig. 11).

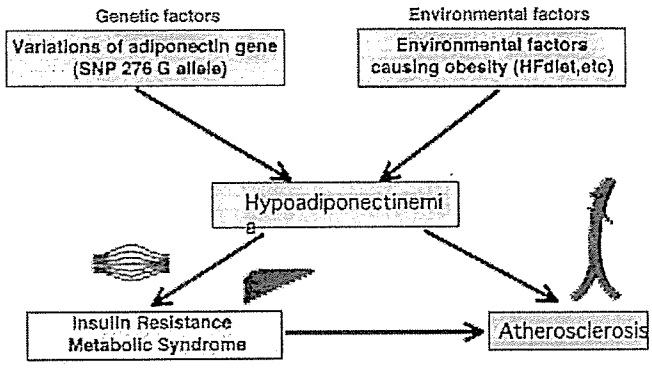

FIG. 11. Adiponectin hypothesis for insulin resistance, metabolic syndrome, and atherosclerosis. Reduced adiponectin levels can be caused by genetic factors such as SNP 276 in the adiponectin gene itself. Reduced adiponectin levels can also be caused by lifestyle changes causing obesity such as a HF diet. Both functional and genetic studies on adiponectin strongly suggest that reduced adiponectin levels play a causal role in the development of insulin resistance, type 2 diabetes, and atherosclerosis (101).

X. Adiponectin and Adiponectin Receptors as Therapeutic Targets

According to our adiponectin hypothesis, a therapeutic strategy for type 2 diabetes, metabolic syndrome, and cardiovascular diseases may include the up-regulation of plasma adiponectin, up-regulation of adiponectin receptors, or the development of AdipoRs agonists.

A. Up-regulation of plasma adiponectin

Insulin sensitizer PPARγ agonists have been shown to increase adiponectin levels in mice (31) and humans (84), as well as in 3T3L1 adipocytes *in vitro* (31). These effects seem to be associated with small-sized adipocytes (39), adipocytes differentiation (85, 86), direct transcriptional activation of genes via peroxisome proliferator response element (87–91), and increased insulin action (92). Interestingly, both PPARγ agonists and adiponectin have been shown to increase insulin sensitivity and ameliorate atherosclerosis. To test whether the PPARγ agonist-mediated improvement in insulin sensitivity and/or amelioration of atherosclerosis was dependent on adiponectin is very important, and thus it is very interesting to see the effects of PPARγ agonists in adiponectin knockout mice.

B. Up-regulation of adiponectin receptors and development of AdipoRs agonists

The evidence described in this review indicates that reductions in plasma adiponectin levels and adiponectin receptors may play major roles in the development of insulin resistance, type 2 diabetes, metabolic syndrome, and cardiovascular diseases that are linked to obesity. With this in mind, one therapeutic strategy may be to up-regulate plasma adiponectin levels, which has already been discussed. The other strategy may be to up-regulate adiponectin receptors or to stimulate adiponectin receptors using small molecule agonists. We would like to introduce two interesting examples of attempts to develop such drugs.

Dr. Staels' group reported that adiponectin receptors are expressed in human macrophages and that their expression levels may be regulated by agonists of the nuclear receptors PPARα, PPARγ, and liver X receptor (93).

Osmotin is a pathogenesis-related (PR)-5 family of plant defense proteins that induces apoptosis in the yeast. Dr. Bressan's group at Purdue University isolated and selected yeast clones that exhibited hypersensitivity to osmotin, sequenced their cDNA inserts, and found that PHO36/YOL002c, the yeast homolog of AdipoR, is a receptor for osmotin (94) (Fig. 12).

X-ray crystallographic studies revealed that both globular adiponectin and osmotin consist of antiparallel β-strands arranged in the shape of a β-barrel. The domain I (lectin-like domain) of osmotin can be overlapped with adiponectin, suggesting that the two proteins share the lectin-like domain (94) (Fig. 12).

Interestingly, osmotin could activate AMP kinase in C2C12 myocytes. More importantly, suppression of AdipoRs expression by siRNA markedly reduced phosphorylation of AMP kinase induced by osmotin. These data suggest that osmotin activates AMP kinase via AdipoRs in mammalian C2C12 myocytes (94).

Osmotin is a member of a large PR-5 protein family, which is both ubiquitous (fruits and vegetables, *etc.*) and diverse. PR-5 proteins are also extremely stable and may remain active even when in contact with the human digestive or respiratory systems. Osmotin, which is a ligand for the yeast homolog of AdipoR (PHO36), activates AMP kinase via AdipoR in C2C12 myocytes. These data raise the possibility that further research examining similarities in adiponectin and

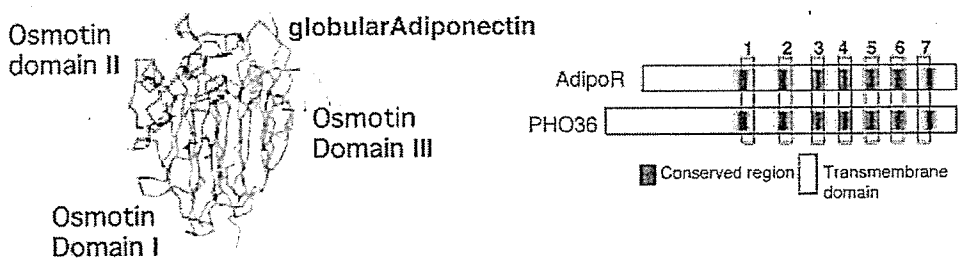

FIG. 12. Osmotin, which is a ligand for the yeast homolog of AdipoR (PHO36), activates AMP kinase via AdipoR in C2C12 myocytes. Osmotin is a member of a large PR-5 protein family, which is both ubiquitous (fruits and vegetables, *etc.*) and diverse. PR-5 proteins are also extremely stable and may remain active even when in contact with the human digestive or respiratory systems. These facts raise the possibility that further research examining similarities in adiponectin and osmotin may facilitate the development of potential adiponectin receptor agonists (94).

osmotin may facilitate the development of potential adiponectin receptor agonists (94).

C. Pleiotropic effects of adiponectin in relation to metabolic syndrome

In this review, we have stated that adiponectin increases insulin sensitivity in the liver and skeletal muscle and that adiponectin also reduces atherosclerosis. In addition to these effects, adiponectin also seems to have pleiotropic effects, particularly in relation to metabolic syndrome. Obesity has been reported to be associated with a higher incidence of certain cancers. Recently, adiponectin was reported to induce antiangiogenesis and antitumor activity via caspase-mediated endothelial cell apoptosis (95). Moreover, fatty liver and/or liver fibrosis are often associated with metabolic syndrome. Adiponectin was reported to alleviate alcoholic and nonalcoholic fatty liver diseases (96, 97) and liver fibrosis (98) in mice. Furthermore, it is possible that adiponectin stimulates insulin secretion and/or regulates energy homeostasis (99, 100). Further studies will be needed to determine the physiological and pathophysiological roles of AdipoR1 and AdipoR2 in these actions.

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