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ACRP30, a new hormone controlling fat and glucose metabolism

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Abstract

Adipocyte complement-related protein of 30 kDa (ACRP30) is a secreted serum protein expressed exclusively in differentiated adipocytes. Recent studies have indicated that its expression and serum levels are reduced in humans and animals with obesity and insulin resistance. Metabolic studies have demonstrated a role for ACRP30 in the regulation of glucose and lipid homeostasis. This review will describe the current literature on the biochemistry of ACRP30 and its physiological functions. We will also discuss issues that are relevant to the directions of future research. © 2002 Elsevier Science B.V. All rights reserved.

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1. Introduction

In recent years, increased understanding of the functions of adipocyte-secreted proteins has challenged the perception of adipose tissue as a mere fat storage depot. Proteins secreted by fat cells, including adipsin, tumor necrosis factor-α (TNF-α), and leptin, participate in regulation of diverse biological processes that include immune responses and metabolic homeostasis. In addition, new genetic models, such as lipoatrophic mice, glucose transporter 4 (GLUT4)-deficient mice, and adipose-specific GLUT4-deficient mice, have demonstrated an essential role for adipose tissue in the regulation of whole body energy homeostasis and, in particular, for glucose homeostasis (Abel et al., 2001; Katz et al., 1996; Moitra et al., 1998; Reitman et al., 1999; Tsao et al., 1997). With recent advances in the understanding of its function, ACRP30 can now be added to the list of adipocyte-derived hormones that play important roles in the regulation of glucose and lipid metabolism and body weight.

2. Purification and cloning of ACRP30

ACRP30 was first identified as a protein expressed and secreted by differentiated murine 3T3-L1 adipocytes (Scherer et al., 1995) using a cDNA subtractive library enriched for mRNAs upregulated during adipocyte differentiation. (Baldini et al., 1992). It was found to be present in abundant quantity in serum (Scherer et al., 1995). It was independently cloned as AdipoQ by differential display of murine mRNAs among non-adipogenic 3T3-C2 fibroblasts, undifferentiated 3T3-F442A preadipocytes, and differentiated 3T3-F442A adipocytes (Hu et al., 1996). The human homologue of ACRP30 was originally identified as the most frequently encountered transcript in female human adipose tissue when sequencing random cDNA library clones (Maeda et al., 1996) and, thus, given the name APM1 (adipose most abundant gene transcript 1). Independently, Nakano et al. (1996) purified ACRP30 protein directly from human plasma while searching for serum proteins with affinity for gelatin–cellulose resins, similar to collagen-binding proteins.

Primary sequence of ACRP30 is shown in Fig. 1. ACRP30 contains a signal peptide at the N-terminus followed by a short hypervariable region with no homology among different species. A collagenous region containing 22 perfect Gly-X-Pro or Gly-X-Y repeats follows. The C-terminal half of the protein contains a globular domain with sequence homology to C1q. N-terminal sequencing of human ACRP30 purified

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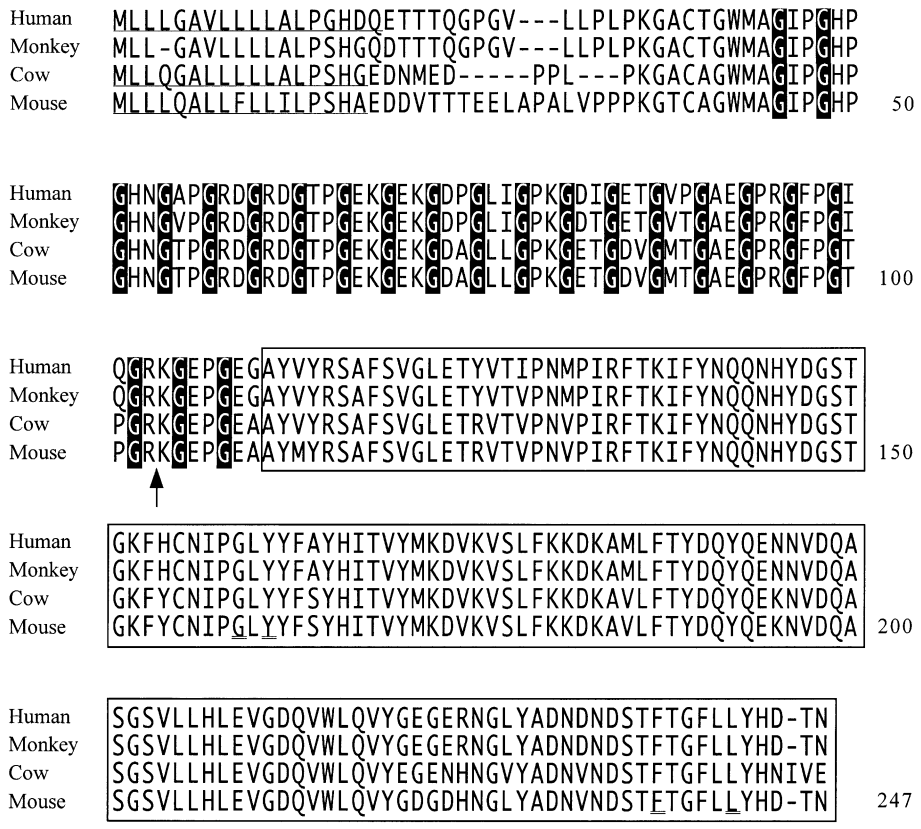

Fig. 1. ACRP30 primary sequence, domain structure, and alignment among human, monkey, bovine, and mouse orthologs. ACRP30 contains four structural domains based upon its primary sequence. At the very N-terminus is a signal sequence (underlined) followed by a region with little similarity among the species represented. The 22 periodic glycine residues defining the collagenous domain are shaded. The globular domain of ACRP30 (boxed) is located at the C-terminus. Amino acid residue numbers to the right refer to the mouse sequence only. Double underlined are key residues conserved between ACRP30 and the TNF-α family of cytokines; these residues are critical for maintaining the three-dimensional structure of ACRP30. The arrow indicates one of the trypsin cleavage sites that generated gACRP30. The others are distributed in the collagenous region.

by gelatin chromatography indicates that residues 1–18 of the deduced amino acid sequence is most likely a signal peptide cleaved during cellular processing (Nakano et al., 1996). The corresponding signal peptide in mouse ACRP30 stretches from amino acids 1–17 (Yoda et al., 2001) (Fig. 1). As can be seen in Fig. 1, the sequence of ACRP30 is highly conserved among several mammalian species.

3. Biochemical and physical characteristics of ACRP30

ACRP30 belongs to a family of proteins that contain sequences homologous to the C1q globular domain (Kishore and Reid, 1999, 2000). To date, 23 members of this family have been identified and are found in species as distant as Xenopus and zebrafish. The family can be broadly divided into two groups based upon the presence or absence of a collagenous domain. Members containing a collagenous domain include ACRP30, C1qA, B, and C chains, saccular collagen (an inner ear-specific protein), hibernation-related protein HP-20, 25, and 27, type VIII and type X collagens, CORS26, and EMILIN. The ones lacking a collagen domain include multimerin and the precursors of cerebellins 1 and 3. With the exception of C1q, the molecular functions of this class of proteins are not well understood though they are implicated in a wide variety of biological processes from regulation of energy metabolism in hibernating animals, such as chipmunks (HP-20, 25, 27) (Kojima et al., 2000; Kondo and Kondo, 1992; Takamatsu et al., 1993), to skeletal and cartilage development (CORS26) (Maeda et al., 2001b).

The crystal structure of the globular portion of ACRP30 (aa 111–247) was determined at 2.1-Å resolution (Shapiro and Scherer, 1998). It revealed a homotrimeric structure with a central hydrophobic core formed by the interface of the three individual monomers. Two of the three monomers have a 10-strand jelly roll topology and one monomer of the trimer unit is folded somewhat differently (one of the 10 strands became a loop). The most surprising finding is that ACRP30 is a structural homologue of the TNF-α family of trimeric cytokines. Although they share very little primary sequence homology, sequence alignment of TNF-α family members and ACRP30 family members has revealed four conserved residues that are crucial in maintaining the architecture of the fold (Fig. 1).

Analysis of human and mouse plasma by size exclusion gel chromatography revealed that ACRP30 forms multiple

species of different apparent molecular weights (Nakano et al., 1996; Scherer et al., 1995; Yoda et al., 2001). Fractionation of mouse serum by sucrose velocity gradient centrifugation revealed a 90-kDa species that corresponds to a ACRP30 trimer and an approximately 300-kDa species that may represent a homononamer or homododecamer (Scherer et al., 1995). A portion of ACRP30 in plasma could be isolated by gelatin–cellulose affinity chromatography (Nakano et al., 1996; Yoda et al., 2001). The portion of human and mouse ACRP30 with affinity toward the gelatin–cellulose matrix migrated as a 420-kDa molecular weight species in gel filtration chromatography (Nakano et al., 1996; Yoda et al., 2001). The same group observed a second immunoreactive protein of 300-kDa apparent molecular weight in both human and mouse plasma. However, this form did not bind to the gelatin–cellulose matrix. Obviously, further studies are required to fully understand the formation of trimeric and multimeric complexes under physiological conditions. In particular, we do not know whether these are high molecular weight homo-oligomers of ACRP30 or are hetero-oligomers of ACRP30 with other unknown protein(s). In addition, the question of whether these forms differ in their biological activity has not been addressed.

Hydroxylation of proline and lysine residues is a common feature of collagens. Although it has not been rigorously tested, it seems likely that the N-terminal collagenous domain of ACRP30 is similarly modified. Although not required for triple helix formation, proline hydroxylation can significantly increase the stability of the triple helix structure of collagen-like peptides (Frank et al., 2001; Sakakibara et al., 1973). Hydroxylation of the collagenous ACRP30 domain may stabilize multimerization in a similar manner.

ACRP30 contains two potential sites for N-linked glycosylation: one in the collagen segment, the other in the globular domain (Sato et al., 2001; Scherer et al., 1995). However, in metabolic pulse-chase experiments endo-*N*-acetylglucosaminidase H treatment did not affect the gel mobility of ACRP30 (Scherer et al., 1995), speaking against the presence of *N*-glycosylation. Other groups have confirmed this finding. Recently, however, bovine and mouse ACRP30 was reported to contain *O*-linked glycan structures containing α2,8-linked disialic acid residues (Sato et al., 2001). In contrast to proteins, such as neural cell adhesion molecule (NCAM) that contain a high number of sialic acid residues, bovine ACRP30 on average contains only 1.4 sialic acid residue/molecule (Sato et al., 2001). Human and mouse C1q are heavily glycosylated at hydroxylysine residues with the disaccharide glucosyl galactose and the monosaccharide galactose (Shinkai and Yonemasu, 1979; Yonemasu et al., 1981). It is likely that other members of the C1q-like globular domain family that also contain collagen domains are glycosylated as well. *O*-linked glycosylation is thought to stabilize triple helix collagen structures (Bann and Bachinger, 2000; Bann et al., 2000). It is possible the formation of ACRP30 oligomers is aided by glycosylation in the collagen domains. However, the portion of plasma ACRP30 that is glycosylated remains unclear and the functional significance of the glycoside moiety in ACRP30 is unknown.

4. Clinical observations and regulation of circulating ACRP30 levels

The first indications that ACRP30 may participate in energy homeostasis came from the observation that its expression is decreased in obese and leptin-deficient *ob*/*ob* mice as well as in a very small cohort of obese humans (Hu et al., 1996). This observation was extended to *db*/*db* mice fed a high-fat diet (Yamauchi et al., 2001). It held true for monkeys (Hotta et al., 2001) and also for larger cohorts of humans (Arita et al., 1999; Hotta et al., 2000; Yang et al., 2001). Using enzyme-linked immunosorbent assay, Arita et al. showed a significant negative correlation between body mass index (BMI) and ACRP30 plasma levels in Japanese men and women. The mean plasma ACRP30 concentration was 8.9 μg/ml for non-obese subjects and 3.7 μg/ml for obese subjects (Arita et al., 1999). The same study revealed two other interesting results. First, human plasma ACRP30 levels varied greatly among non-obese subjects, ranging from 1.9 to 17 μg/ml (Arita et al., 1999). Second, female subjects had higher plasma ACRP30 levels than males. Subsequently, another study extended the negative correlation observed in Japanese subjects between plasma ACRP30 level and body fat content to Caucasian and Pima Indian populations (Weyer et al., 2001).

The same group went on to study human plasma ACRP30 levels relative to additional physiological parameters. They compared ACRP30 levels in obese subjects with similar BMI, dividing them into three groups: nondiabetic subjects, diabetic subjects with coronary artery disease, and diabetic subjects without coronary artery disease. They found that obese diabetic people had even lower plasma ACRP30 levels than nondiabetic obese subjects (Hotta et al., 2000). Obese diabetic subjects with coronary artery disease had the lowest ACRP30 levels and was the case both for men and women (Hotta et al., 2000). Similar to an earlier study, women again had higher plasma ACRP30 levels than men (Hotta et al., 2000). They also showed that the plasma ACRP30 levels changed very little during the course of the day and did not follow any significant circadian rhythm.

Separate studies also found that ACRP30 concentration in plasma correlated negatively with fasting insulin levels in Japanese (Hotta et al., 2000), Caucasian (Weyer et al., 2001), and Pima Indian (Weyer et al., 2001) populations. In addition, human plasma ACRP30 levels negatively correlated with plasma triglyceride concentration (Hotta et al., 2000) as well as fasting (Hotta et al., 2000) and postprandial (Weyer et al., 2001) plasma glucose concentration. One study also showed that the level of plasma ACRP30 correlated positively with

insulin-stimulated whole body glucose disposal (Weyer et al., 2001). It is noteworthy that the most significant association found was the negative correlation between plasma ACRP30 levels and insulin resistance and hyperinsulinemia (Weyer et al., 2001).

A recent study employing rhesus monkeys provided a detailed picture of how ACRP30 levels change during the development of obesity and diabetes (Hotta et al., 2001). Rhesus monkeys are prone to develop obesity spontaneously; development of obesity is accompanied by progressive worsening of insulin sensitivity, elevated fasting insulin levels, and eventual hyperglycemia (Hansen and Bodkin, 1986). In a prospective longitudinal study of rhesus monkeys, progression of obesity and insulin resistance was associated with an ever-decreasing concentration of plasma ACRP30 (Hotta et al., 2001). A striking linear correlation was also observed between insulin-stimulated glucose disposal and plasma ACRP30 levels (Hotta et al., 2001). However, the true value of this longitudinal study was the observation that plasma ACRP30 level started to drop at an early phase, prior to the onset of frank hyperglycemia, glucose intolerance, and maximal level of obesity. Equally important was the observation that plasma ACRP30 levels remained decreased during the very late phase in which fasting insulin levels have significantly dropped and the monkeys suffered from frank hyperglycemia as well as fat pad and body weight loss (Hotta et al., 2001). These results suggest that a decrease in plasma ACRP30 level may contribute causally to development of obesity and type II diabetes mellitus.

During the progression toward diabetes, the decrease of plasma ACRP30 in rhesus monkeys contrasted sharply with an increase in the concentration of plasma leptin (Hotta et al., 2001). Elevation of leptin levels in the plasma most likely reflects development of leptin resistance, a well-documented phenomenon in obese animals (Ahima and Flier, 2000). Since ACRP30 levels are actually decreased in obese and insulin-resistant animals, pharmacological use of ACRP30 to control obesity and diabetes might not be thwarted by resistance to ACRP30.

In contrast to the situation with obesity and insulin resistance, ACRP30 levels rise with weight loss (Hotta et al., 2000; Yang et al., 2001), caloric restriction (Berg et al., 2001), cold exposure (Yoda et al., 2001), and, interestingly, restoration of insulin sensitivity by thiazolidinedione treatment (Berg et al., 2001). In a limited cohort of nondiabetic and diabetic Japanese subjects, plasma ACRP30 levels increased by 42% and 65%, respectively, following weight reduction (Hotta et al., 2000). In the same study, plasma leptin levels decreased by 58% and 46% for nondiabetic and diabetic subjects, respectively (Hotta et al., 2000). Similarly, mice whose caloric intake had been restricted to 60% of ad libitum levels exhibited reduced body weight and increased plasma ACRP30 concentration (Berg et al., 2001). When mice were placed at 4 °C, their plasma ACRP30 levels increased from 6 to 24 h, then gradually returned to normal levels (Yoda et al., 2001).

Since weight loss is associated with improved insulin action, restoration of insulin sensitivity in insulin-resistant animals may also result in enhanced ACRP30 levels. Indeed, treatment of *db*/*db* mice with thiazolidinediones, a PPARγ agonist and insulin sensitizer, dramatically increased plasma ACRP30 levels and ameliorated the severity of hyperglycemia and insulin resistance (Berg et al., 2001; Yamauchi et al., 2001). Treating normal C57BL/6J mice kept on a high-fat diet with thiazolidinediones also increased plasma ACRP30 levels and white adipose tissue ACRP30 mRNA (Yamauchi et al., 2001) (Combatsiaris et al. ADA, 2001, Abstract 1118-P). Thiazolidinedione treatment of insulin-resistant humans similarly increased the plasma ACRP30 concentration (Maeda et al., 2001a). In contrast to thiazolidinediones, PPARα agonists or metformin had no effect on plasma ACRP30 levels (Combatsiaris et al. ADA, 2001, Abstract 1118-P). The studies described above suggest that the improved insulin sensitivity following thiazolidinedione treatment may, at least in part, be mediated by increasing ACRP30 levels. However, additional studies are required since a known side effect of treatment with thiazolidinediones is increased body weight, presumably caused by induction of adipogenesis. This was also observed in the above studies and stands in conflict with the reported weight-reducing effect of ACRP30 treatment (Fruebis et al., 2001).

While thiazolidinedione treatment increased ACRP30 levels, the opposite was seen for TNF-α, which is implicated in causing insulin resistance. TNF-α reduced the expression of ACRP30 in differentiated murine 3T3-L1 and primary human adipocytes (Kappes and Loffler, 2000; Maeda et al., 2001a). TNF-α is produced by adipose tissue and its expression level is significantly elevated in obesity (Hotamisligil et al., 1993, 1995). This raises the important question of whether the increase in TNF-α directly leads to down regulation of ACRP30 mRNA and protein in obese subjects and whether this is a mechanism by which TNF-α induces insulin resistance. Supporting this hypothesis, lipoatrophic mice that lack adipose tissue develop hyperinsulinemia and insulin resistance similar to obese animals (Moitra et al., 1998; Shimomura et al., 1999; Yamauchi et al., 2001). Both animal models of insulin resistance and diabetes exhibit low plasma ACRP30 levels.

5. Genetic evidence for involvement of ACRP30 in diabetes and obesity

The gene for human ACRP30 spans 17 kb on chromosome locus 3q27 (Saito et al., 1999a; Takahashi et al., 2000). The mouse ACRP30 gene maps to the telomere region of chromosome 16, an area syntenic to human 3q27 (Das et al., 2001). Both mouse and human genes have a similar genomic structure that include three exons, with the start codon in exon 2 and stop codon in exon 3 (Das et al., 2001; Saito et al., 1999a; Takahashi et al., 2000). A number of groups have conducted promoter studies on the human and mouse

ACRP30 gene (Das et al., 2001; Saito et al., 1999a,b; Schaffler et al., 1998, 1999). Although thiazolidinediones can increase ACRP30 mRNA levels, no classical PPARγ-binding sites were found in ACRP30 promoter. Among the putative regulatory elements identified, three CCAAT/enhancer-binding protein (C/EBP) sites were found. These play an important role in the differentiation of preadipocytes to adipocytes and can activate PPARγ. Together, C/EBP and PPARγ are the two major groups of transcription factors that play an important role during adipocyte differentiation. Therefore, while no classical PPARγ-binding site exists in ACRP30 promoter, indirect activation by PPARγ may occur after treatment of cells with thiazolidinediones via the C/EBP sites.

In a recently performed genomewide scan done in a large group of human subjects, two quantitative trait loci (QTLs) were identified that influence the phenotype of Metabolic Syndrome (insulin resistance, obesity, hypertension, and coronary artery disease) (Kissebah et al., 2000). The most strongly linked QTL was on chromosomal location 3q27. The traits that had highly significant Lod scores at 3q27 included BMI, body weight, and fasting insulin levels. Although this region contains a large number of genes (possibly over 100), one of them is ACRP30. An independent study also mapped 3q27 as a locus for susceptibility of early onset diabetes in French Caucasians (Vionnet et al., 2000).

6. Potential roles of ACRP30 in metabolism

Three recently published papers described for the first time important metabolic effects of ACRP30. Fruebis et al. (2001) showed that when injected into mice ACRP30, and more potently the globular head region of ACRP30 (gACRP30), led to a number of effects. The highly active gAcrp30 was prepared in vitro as a proteolytic cleavage product of bacterially expressed and purified ACRP30. gAcrp30 treatment accelerated the oxidation of non-esterified fatty acids by muscle. This effect was accompanied by a decrease in plasma glucose level independent of insulin and glucagon. In addition, chronic gACRP30 treatment resulted in reduction of body weight independent of food intake. This was the first direct in vivo evidence supporting the hypothesis that ACRP30 contributes to the control of energy homeostasis and that it exerts its effect primarily at the peripheral level. An increase in free fatty acid oxidation caused by gACRP30 occurred both in isolated muscle as well as in cultured C2C12 skeletal muscle cells; it was not seen in cultured hepatocytes. Additional experiments indicated the presence of a fragment of ACRP30 containing the globular head in human plasma. It was suggested that similar to precerebellin—another member of the C1q protein family—ACRP30 might be an inactive or weakly active precursor of the active globular domain fragment.

These findings were largely confirmed and extended by two other papers (Berg et al., 2001; Yamauchi et al., 2001). Berg et al. (2001) reported that elevating the plasma level of ACRP30 two- to threefold by intraperitoneal injection of recombinant ACRP30 transiently decreased basal plasma glucose levels without increasing plasma insulin concentrations. The glucose lowering effect of ACRP30 was observed at 4 h after injection and plasma glucose returned to normal levels by 6 h following ACRP30 injection. This effect was seen in *ob*/*ob* mice, a model of type II diabetes, as well as in NOD mice, a type I diabetes model. In both diabetic mouse models after ACRP30 injection, glucose levels remained lower for a longer period of time than with normal mice treated similarly. In isolated mouse hepatocytes, ACRP30 increased the ability of sub-physiological levels of insulin (35 pM) to suppress gluconeogenesis, indicating ACRP30 might act as insulin-sensitizing protein. ACRP30 had minimal effects on hepatocyte glucose production in absence of insulin. Importantly, the concentrations of ACRP30 used in these experimental conditions were very much in line with physiological concentrations. Furthermore, an increase in plasma glucagon was seen in ACRP30 treated mice, most likely a response to decreased plasma glucose levels. Serum triglyceride levels in ACRP30-treated animals were lower compared to non-treated control animals indicating that, in addition to its effect on glucose metabolism, fat metabolism was also accelerated. Lastly, Berg et al. reported that rosiglitazone, a member of the insulin-sensitizing pharmacological agents thiazolidinediones, effectively increased ACRP30 secretion in vivo in *db*/*db* mice and speculated that ACRP30 might be the mediator of thiazolidinedione action.

Yamauchi et al. (2001) demonstrated improved insulin sensitivity, decreased storage of triglycerides in liver and muscle, and increased expression of proteins in muscle involved in both fatty acid combustion and dissipation upon long-term treatment (12 days) with ACRP30. The authors observed these effects using bacterially expressed full-length protein and globular head protein (gACRP30), with the latter being much more active. Insulin resistance in lipoatrophic mice was partially reversed by leptin or ACRP30 treatment alone, but was completely reversed when both proteins were infused. In addition, hyperglycemia and elevated plasma triglyceride and free fatty acid levels were markedly improved upon treatment with ACRP30 and even more so with very low doses of gACRP30. Amelioration of insulin resistance in lipoatrophic mice by ACRP30 or gACRP30 was accompanied by reduced liver and muscle triglyceride content.

Furthermore, Yamauchi et al. confirmed the induction of ACRP30 expression in *db*/*db* mouse white adipose tissue and in 3T3-L1 adipocytes by the thiazolidinedione rosiglitazone. Treatment with gACRP30 led to increased expression of fatty acid translocase CD36, acyl CoA oxidase, UCP2, and PPARα in muscle tissue of lipoatrophic mice. These are genes whose enhanced expression may explain the increased rate of muscle lipid metabolism and decreased tissue triglyceride storage. Additional effects of long-term

gACRP30 treatment in lipoatrophic mice included increased phosphorylation of the insulin receptor, insulin receptor substrate 1 (IRS-1), and Akt in skeletal muscle following insulin stimulation, possibly due to decreased triglyceride content. Treatment with ACRP30 or gACRP30 also ameliorated insulin resistance in *db*/*db* and yellow agouti KKA^y mice. This was accompanied by increased fatty acid oxidation rate and acyl-CoA oxidase activity in muscle, and, similar to the effects on lipoatrophic mice, decreased muscle and liver triglyceride content.

The finding that thiazolidinedione treatment increased ACRP30 levels suggests that ACRP30 may be a mediator of the insulin-sensitizing effect of thiazolidinedione. While intriguing, this does not explain why thiazolidinedione treatment leads also to increased body fat and body weight gain, which by itself should reduce ACRP30 expression. One possible explanation could be that thiazolidinediones promote adipogenesis, whereas body fat regulation can also take place solely by storage of fat in existing adipocytes. Second, the target tissue of thiazolidinediones and of ACRP30 might not be the same, leading to different effects.

The three different reports described above contained many similar findings. For example, all three papers described reduction of serum triglyceride levels following ACRP30 treatment. The decrease in serum triglyceride level stands seemingly in contradiction to the finding in Berg et al. that muscle and liver triglyceride levels did not decrease as much in treated animals as in controls. However, this observation agrees with the finding in Fruebis et al. (2001) that after acute treatment, muscle triglyceride storage actually increases before the levels decrease under chronic treatment. Indeed, Yamauchi et al. reported that long-term treatment with ACRP30 reduced liver and muscle triglyceride content.

There were apparent disagreements among these three reports that most likely reflected differences in experimental systems. Fruebis et al. (2001) reported that full-length ACRP30 injected into mice elicited minimal or no effects on plasma glucose, free fatty acid, and triglyceride concentrations. The differences between the results in Fruebis et al. and those in Berg et al. were attributed to the source of recombinant ACRP30 (Berg et al., 2001). In Fruebis et al. ACRP30 was purified from bacteria expressing ACRP30, whereas Berg et al. expressed ACRP30 in mammalian cells and, thus, the protein could have been modified by prolyl hydroxylation and/or glycosylation. However, the experiments in Berg et al. (2001) also used at least 15-fold more ACRP30 than those in Fruebis et al. (2001) (28–84 μg/g body weight vs. 25–50 μg/mouse).

Berg et al. described the plasma glucose lowering effect of ACRP30 and the synergistic effects of ACRP30 and insulin in hepatocytes. However, they did not see an effect when just the globular domain of ACRP30 was administered (Berg et al., 2001). In contrast, Yamauchi et al. (2001) reported a significantly higher potency of their globular construct of ACRP30 in reversing insulin resistance, a finding that is in agreement with the results in Fruebis et al. (2001).

This may be explained by gACRP30 and full-length ACRP30 having different physiological functions. Whereas gACRP30 acts on muscle to stimulate fatty acid oxidation, it may not act on liver to inhibit glucose production. On the other hand, ACRP30 may be able to act on both liver and muscle, albeit only at high concentrations. Long-term ACRP30 treatment of lipoatrophic or obese mice reduced triglyceride content in both liver and muscle (Yamauchi et al., 2001). Although ACRP30 facilitated reduction of hepatocyte glucose production by insulin, injection of ACRP30 into mice had no effect on muscle and liver glycogen content (Berg et al., 2001). These results suggest that the primary site of its plasma glucose lowering effect is liver, not muscle. However, it is important to note that because all experiments in this study were done in the fasted state, effects on glucose metabolism were mainly on gluconeogenesis and not on peripheral use of glucose as energy substrate.

7. Additional functions of ACRP30

The expression and genetic data described above strongly suggest a regulatory role for ACRP30 in body weight maintenance and insulin action. In addition, there are several functional studies implicating ACRP30 in anti-atherogenic and anti-inflammatory processes. A group led by Drs. Funahashi and Matsuzawa first described accumulation of ACRP30 immunoreactivity in balloon-injured arterial walls (Okamoto et al., 2000). They went on to show the functional significance of their initial finding in endothelial cells and in macrophages. Using recombinant ACRP30 purified from *Escherichia coli* following denaturation, solubilization, and refolding procedures, they showed that an 18-h treatment of human aortic endothelial cells with ACRP30 attenuated the induction of adhesion molecules expression by TNF-α (Ouchi et al., 1999). It was further shown that the attenuation of adhesion molecule expression occurred through cAMP-dependent inhibition of NF-κB signaling (Ouchi et al., 2000). The same group also reported that ACRP30 could inhibit phagocytosis and LPS-induced TNF-α production in human monocyte-derived macrophages (Yokota et al., 2000). However, ACRP30-mediated inhibition of phagocytosis may not be specific since it can be blocked by neutralizing antibody against a receptor for C1q, C1qRp (Yokota et al., 2000). Collectively, these studies suggest that ACRP30 has anti-inflammatory properties antagonistic to TNF-α. In addition to being anti-inflammatory, ACRP30 may also have anti-atherogenic properties. Long-term (2–3 days) treatment of human macrophages with ACRP30 reduced cholesteryl ester and lipid accumulation, possibly by decreasing expression of the class A macrophage scavenger receptor (Ouchi et al., 2001). Through this mechanism, ACRP30 may suppress the transformation of macrophages to foam cells.

8. Challenging issues for the future

At this point, a sizable body of literature on ACRP30 biology has been accumulated supporting the view that ACRP30 contributes to the regulation of lipid and glucose metabolism and, thus, can play a critical role in the development of obesity and diabetes. This hypothesis can be confirmed by inactivating ACRP30 using a gene-targeting approach. Another area of interest is to understand how circulating ACRP30 levels may be regulated over the long term since, unlike insulin and leptin, plasma ACRP30 levels remain constant during different times of the day and are not affected acutely by food intake (Hotta et al., 2000). It will also be important to identify the underlying causes responsible for the decline in ACRP30 levels in obese and diabetic states.

A crucial step toward understanding the biology of ACRP30 is determining the active circulating form of the protein capable of mediating the same effects as exogenously supplied recombinant ACRP30. The average plasma ACRP30 concentration is around 5–10 μg/ml, or approximately 50–100 nM of ACRP30 homotrimer. Since most ligand–receptor complexes have Kds in the low nanomolar range, the amount of ACRP30 homotrimer in the serum will likely overwhelm all available receptors on the cell surface. Three potential models of ACRP30 activation may account for this apparent paradox. First, we have demonstrated that globular portion of ACRP30 alone can stimulate lipid oxidation and lowering plasma glucose without the presence of the collagenous domain (Fruebis et al., 2001). We have also identified in human plasma a C-terminal fragment of ACRP30 that is at much lower abundance than the full-length protein (Fruebis et al., 2001). Thus, it is possible that full-length ACRP30 is inactive and that proteolytic cleavage(s) is required to yield the active globular domain. This hypothesis is supported by a recent study showing that in lipoatrophic mice the C-terminal globular domain of ACRP30-ameliorated insulin resistance more potently than the full-length protein (Yamauchi et al., 2001). Alternatively, ACRP30 forms higher-order complexes that probably contain 12 or more monomers (Scherer et al., 1995). Through cooperative binding or receptor clustering, larger ACRP30 homo- or hetero-oligomers may achieve specific and high affinity binding to receptors that cannot be achieved by ACRP30 homotrimers (Berg et al., 2001). The same rationale also applies to fragments of the full-length protein; large multimers might not be able to bind efficiently to receptors. A third model of ACRP30 action incorporates elements of the two previous ones. Since most of the circulating ACRP30 forms multimers, the concentration of the trimer form may be low enough for it to be the active form that can interact with the putative receptor. The crystal structure of the ACRP30 globular domain showed that it assembles into homotrimers (Shapiro and Scherer, 1998). Structurally, there may be little difference between the globular ACRP30 trimer and full-length trimer. Thus, this model is consistent with the results of previous studies demonstrating the effectiveness of globular ACRP30 in weight loss and insulin resistance amelioration.

One issue that arose from the various and sometimes seemingly conflicting studies reviewed here is the primary physiological role of ACRP30. Is it body weight control or insulin sensitivity? Based upon the very limited amount of literature available, plasma ACRP30 levels correlated better with insulin sensitivity than body weight or adiposity (Hotta et al., 2000, 2001; Weyer et al., 2001). However, obesity and type II diabetes are tightly linked physiologically and cannot be studied independent from each other. A more relevant question may be whether the principal action of ACRP30 is on glucose or on lipid metabolism. Current data show that ACRP30 can influence both independently at a primary level. Insulin's role on lipolysis regulation does not depend upon its action on glucose uptake, and vice versa, and signaling pathways activated by ACRP30 may lead to both fatty acid oxidation and glucose production. Besides its impact on body weight maintenance, oxidation of fatty acids may influence muscle insulin sensitivity by regulating muscle triglyceride content. Conversely, the ability of ACRP30 to reduce hepatic glucose production may prevent development of hyperinsulinemia, which in turn will discourage triglyceride deposition and development of obesity. It should simply suffice to conclude that ACRP30 plays an important role in the regulation of both body weight and insulin sensitivity.

Irrespective of the mode of its primary action, ACRP30 holds great promise as a pharmacological agent to treat dysregulation of body weight and glucose homeostasis. Unlike potential cellular targets of pharmacological intervention, ACRP30 is a circulating hormone and its action can be readily achieved by injection of recombinant forms of the protein. Either by blocking excess hepatic glucose production or stimulating fatty acid oxidation in muscle, ACRP30 may represent a novel approach to control type II diabetes mellitus and obesity.

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