FOCUS

Adiponectin and its Metabolic Roles

Excess accumulation of body fat increases the risk of developing hypertension, diabetes, heart disease, stroke, and other illnesses. The body mass index (BMI) is a commonly used standard for assessing weight-related health risks. It is calculated by dividing the weight of the body in kilograms by the square of its height in meters (kg/m^2). Using this index, individuals with a BMI of 27-30 kg/m^2 are considered overweight, whereas adult obesity is defined as having a BMI of greater than 30 kg/m^2. According to the Centers of Disease Control (CDC), the prevalence of obesity among U.S. adults has nearly doubled since 1980, and currently more than 27% of the adult population is considered obese (1). The underlying physiological, psychological, and social causes for this epidemic are complex and not fully understood. Yet, from a nutritional point of view, obesity is clearly the end result of a sustained imbalance between food intake and energy expended. The extra caloric input is primarily converted into glycogen and triglycerides (fat), which are stored in the liver and adipose tissue, respectively. In response to changing energy demands hepatic glycogen is converted into glucose, whereas the adipose tissue transfer energy by secreting free fatty acids (FFAs). Normally, circulating FFAs are consumed by the muscle, the main tissue that utilizes this type of energy. However, if high levels of FFAs remain in circulation, they may cause severe health problems, including insulin resistance and atherosclerosis. Atherosclerosis, marked by fatty deposits in the inner walls of arteries, is the leading cause of illness and death in Western countries. In general, health risks associated with elevated blood FFA levels can be reduced by a combination of low-fat diet and adequate physical activity regimen.

In addition to FFAs, adipose tissue secretes a number of hormonal factors, including Leptin, Resistin, Adipsin, TNF-α, and Adiponectin. These fat-derived hormones, also called adipocytokines or adipokines, play an important role in energy homeostasis by regulating glucose and lipid metabolism. A major breakthrough in understanding obesity-related metabolic disorders was the recent discovery that full-length adiponectin and its truncated derivative, called gAcrp30 or Adipolean, are anti-diabetic and anti-atherogenic proteins. Here we highlight these proteins and their mode of action.

Adiponectin is a secreted protein, produced exclusively by adipocytes. It was independently discovered by four different research groups, and received several names, including adipocyte complement-related protein of 30 kDa (ACRP30), gelatin binding protein of 28 kDa (GBP28), adipose most abundant gene transcript-1 (APM-1), AdipoQ, and adiponectin (table I).

The primary structure of adiponectin consists of three domains, a short N-terminal region with no homology to any known protein domain, a collagen-like domain, and a C-terminal globular domain (Figure 1).

Table 1: Terminology used for adiponectin and its proteolytically-derived fragments

Protein form	Mouse	Human	MW (# of amino acids)
Full length	Acrp30	Adiponectin	25.0 kDa (230)
	AdipoQ	APM-1	25.0 kDa (230)
	OBG3	GBP28	25.0 kDa (230)
Active fragment	gAcrp30 (globular Acrp30)	h gAcrp30/Adipolean*	16.6 kDa (145)
		h gAcrp30/Adipolean Variant**	18.1 kDa (159)

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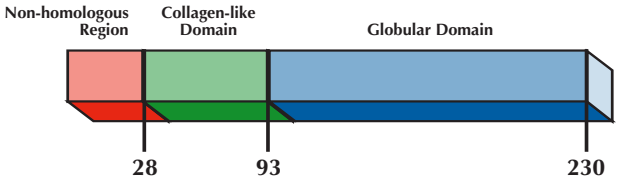
Figure 1: Domain arrangement in the primary structure of Adiponectin.



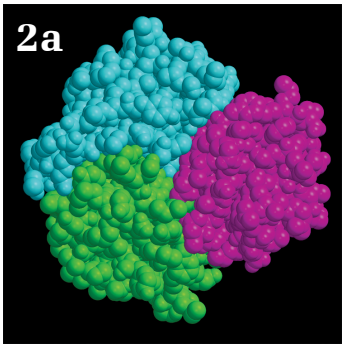
Amino-acid sequence alignment of human and murine adiponectin reveals a 50% sequence identity in the N-terminal region, a 79% identity in the collagen-like domain, and a 91% identity in the globular domain. The high degree of sequence conservation within the globular domain suggests that this domain is important for preserving a vital biological function. (Figure 1)

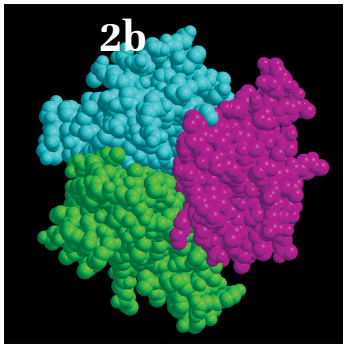
Adiponectin is relatively abundant in humans and rodents, accounting for about 0.01% of total plasma protein. Circulating adiponectin exists in several homo-oligomeric isoforms, including trimers, hexamers, and higher order multimers (2). The biological significance of these isoforms is still unclear, but it has been demonstrated that oligomerization of full length adiponectin is critical for manifestation of its biological activity, and requires post-translation hydroxylation and glycosylation of several lysine residues within the collagenous domain of the protein (3).

Increased adiposity is generally associated with decreased insulin sensitivity along with elevated plasma levels of various adipokines, including leptin, resistin, and TNF-α. In contrast, the plasma levels of adiponectin are decreased under conditions of obesity, insulin resistance, and type 2 diabetes mellitus (adult-onset diabetes) (4). Disruption of adiponectin in mice causes insulin resistance and neointimal formation (thickening of the lining of arteries) (5). Conversely, administration of recombinant adiponectin suppresses hepatic glucose production, and reverses insulin resistance associated with both lipoatrophy and obesity (6, 7).

Adiponectin and its Metabolic Roles continued...

Figure 2: Crystal structures of recombinant murine gAcrp30 and TNF-α





The space-filling models of the 2.1A resolution crystal structure of murine gAcrp30 (13) [figure 2a] and the 1.4Ä resolution crystal structure of murine TNF-α (14) [figure 2b]. The two structures reveal an identical homo-trimeric arrangement, and very similar α-carbon configurations in the all-beta core.

Taken together, these findings indicate that adiponectin plays a protective role against hyper-glycemia, insulin resistance, and atherosclerosis. Preliminary data suggest that adiponectin may fulfill this role, at least in part, by exerting antagonistic effects on TNF-α activity. TNF-α is an inflammatory adipokine, whose elevated plasma levels in obesity have been implicated in the induction of hyperglycemia and insulin resistance (8). Moreover, its ability to suppress adiponectin gene expression (9) might be the primary cause for the decreased levels of adiponectin in obesity. Inversely, adiponectin has been shown to interfere with TNF-α signaling (10) and to inhibit TNF-α expression (11).

Proteolytic processing of adiponectin generates a truncated 16.5 kDa protein, gAcrp30/adipolean, which corresponds to the entire C-terminal globular domain of adiponectin. gAcrp30/adipolean was reported to possess unique pharmacological properties that are not shared with full-length adiponectin (12). It stimulates fatty-acid oxidation in skeletal muscle, and causes profound and sustainable weight loss in mice, without affecting food intake. Treatment of mice with purified gAcrp30 for two weeks significantly decreases the elevated levels of plasma FFAs, caused either by high fat/sucrose diet or i.v. injection of Intralipid (12). Overexpression of gAcrp30 in transgenic mice ameliorates hyperglycemia and insulin resistance induced by high fat diet (11). Furthermore, gAcrp30 protects ob/ob mice from diabetes and ApoE-deficient mice from atherosclerosis (11). The inhibitory effects of gAcrp30 on the accumulation of inflammatory macrophages and lipids in the arterial wall of ApoE-deficient mice have been suggested to result from its ability to suppress the expression of class A scavenger receptor and TNF-α (11). Interestingly, gAcrp30 and TNF-α, which exert opposing effects on the development of atherosclerosis, have unrelated amino-acid sequences, but display highly similar homo-trimeric three-dimensional structures (figure 2) (13).

The signaling receptors used by gAcrp30 and adiponectin have recently been cloned, and named AdipoR1 and AdipoR2 (15). Based on their amino-acid sequences, AdipoR1 and AdipoR2 are structurally-related, and are predicted to contain seven transmembrane domains. AdipoR1 is abundantly expressed in skeletal muscle, whereas AdipoR2 is predominantly expressed in the liver. AdipoR1 is a high-affinity receptor for gAcrp30, but possesses only marginal binding-affinity for full-length adiponectin. In contrast, AdipoR2 is an intermediate affinity receptor for both gAcrp30 and the full-length adiponectin (15). These binding characteristics indicate the existence of two distinct receptor binding-sites within the globular domain of adiponectin, each capable of interacting with either AdipoR1 or AdipoR2. The finding that the AdipoR1 binding-site in full-length adiponectin is inaccessible for receptor binding suggests that the non-globular part of the molecule is masking this site. Proteolytic processing of adiponectin, which generates gAcrp30, removes the non-globular part and exposes the AdipoR1 binding-site.

Since gAcrp30 exhibits biological activity distinct from that of full-length adiponectin, especially in skeletal muscle, quantification of the relative plasma levels of these proteins is important for better characterization of adiponectin-linked metabolic disorders. Toward this end, we have developed highly specific antibodies capable of distinguishing between gAcrp30 and adiponectin. These antibodies are currently being used to develop a highly sensitive ELISA kit for determining the ratio between gAcrp30 and full-length adiponectin.

References

1. Flegal, J.M. et al., J. Amer. Med. Assoc., Vol. 288, 1723-1727 (2002)
2. Tsao, T.S. et al., J. Biol. Chem., Vol. 277, 29359-29362 (2002)
3. Wang, Y. et al., J. Biol. Chem., Vol. 277, 19521-19529 (2002)
4. Hu, E. et al., J. Biol. Chem., Vol. 271, 10697-10703 (1996)
5. Kubota, N. et al., J. Biol. Chem., Vol. 277, 25863-25866 (2002)
6. Yamauchi, T. et al., Nature Med., Vol. 7, 941-946 (2001)
7. Berg, A. H. et al., Nature Med., Vol. 7, 947-953 (2001)
8. Hotamisligil, G.S., Exp. Clin. Endocrinol. Diabetes, Vol. 107, 119-125 (1999)
9. Fasshauer, M. et al., B.B. Res. Commun., Vol. 25, 1084-1089 (2002)
10. Ouchi, N. et al., Circulation, Vol.102, 1296-1301 (2000)
11. Yamauchi, T. et al., J. Biol. Chem., Vol. 278, 2461-2468 (2003)
12. Fruebis, J. et al., Proc. Natl. Acad. Sci. USA, Vol. 98, 2005-2010 (2001)
13. Shapiro, L. and Scherer, P.E., Curr. Biol., Vol. 8, 335-338 (1998)
14. Baeyens, K.J. et al., Acta Cryst. D Biol. Cryst., Vol 55, 772-778 (1999)
15. Yamauchi, T. et al., Nature, Vol. 423, 763-768 (2003)

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