Regulation of angiopoietin-like protein 8 expression under different nutritional and metabolic status

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Abstract. Type 2 diabetes mellitus (T2DM) is a chronic metabolic disease with increasing prevalence worldwide. Angiopoietin-like protein 8 (ANGPTL8), a member of the angiopoietin-like protein family, is involved in glucose metabolism, lipid metabolism, and energy homeostasis and believed to be associated with T2DM. Expression levels of ANGPTL8 are often significantly altered in metabolic diseases, such as non-alcoholic fatty liver disease (NAFLD) and diabetes mellitus. Studies have shown that ANGPTL8, together with other members of this protein family, such as angiopoietin-like protein 3 (ANGPTL3) and angiopoietin-like protein 4 (ANGPTL4), regulates the activity of lipoprotein lipase (LPL), thereby participating in the regulation of triglyceride related lipoproteins (TRLs). In addition, members of the angiopoietin-like protein family are varingly expressed among different tissues and respond differently under diverse nutritional and metabolic status. These findings may provide new options for the diagnosis and treatment of diabetes, metabolic syndromes and other diseases. In this review, the interaction between ANGPTL8 and ANGPTL3 or ANGPTL4, and the differential expression of ANGPTL8 responding to different nutritional and metabolic status during the regulation of LPL activity were reviewed.

Key words: Angiopoietin-like protein 8 (ANGPTL8), Diabetes mellitus, Metabolic status, Nutritional status

Introduction

Triglycerides (TGs) are one of the most important substrates for energy supply. Compared to carbohydrates and proteins, TGs have lower metabolic cost [1, 2]. Therefore, synthesis and storage of TGs are particularly important to maintain the body’s energy balance. Lipoprotein lipase (LPL) is a key rate-limiting enzyme in catalyzing hydrolysis of circulating triglyceride in chyomicrons (CM) and very-low-density lipoprotein (VLDL), the major forms of triglycerides in plasma [3, 4].

LPL hydrolyzes TG from chylomicron and VLDL into free fatty acids, which are absorbed by surrounding tissues such as fat, muscle, and heart [5]. To cope with the energy requirement of the body under different nutritional and metabolic status, TGs in the circulation are mainly processed by LPL and distributed in oxidized tissues (such as muscle) and white adipose tissue (WAT) under capillary endothelial cells [6]. LPL has hydrolytic activity in the form of dimer, and the dissociation of LPL dimer is the key to its spontaneous inactivation [7]. In the fasted state, LPL activity in WAT is down-regulated, while its activity in heart and skeletal muscle is up-regulated to meet the energy needs of the body. In the fed state, LPL activity in WAT is increased, thereby increasing the energy reserve of the body [4, 7]. Therefore, LPL plays an important role in maintaining the energy balance of the body.

Angiopoietin-like proteins (ANGPTLs) are a family of secreted glycoproteins consisting of the N-terminal helix domain and the fibrinogen-like C-terminal domain. ANGPTLs often participate in angiogenesis, stem cell expansion, inflammation, tissue remodeling, and lipid metabolism. Genetic and functional studies have shown that ANGPTL3, ANGPTL4, and ANGPTL8 play important roles in the regulation of LPL activity [8-12].

ANGPTL8 (also called RIFL, lipasin, and betatrophin) consists of 198 amino acids and is about 22 kDa in size. The human ANGPTL8 gene was designated as LOC55908, C19ORF80, or TD26, and located at chromosome 19p13.2. In mice, the gene is located at chromosome 9 and is called Gm6484 or E624219 [13]. Studies have shown that human ANGPTL8 is dominantly expressed in liver, while mouse ANGPTL8 has highest expression levels in liver and white and brown adipose tissues. ANGPTL8 is considered to be an atypical mem-

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cytes [24]. TNF-α is a cytokine that inhibits adipogenesis by many factors, such as glucose, insulin and GLP-1 [13, 18-20]. The expression levels of ANGPTL8 is also regulated by the nutritional status, and its expression is significantly increased in the refeeding state [23].

**ANGPTL8 in Regulation of Lipid Metabolism**

ANGPTL8 is regulated by the nutritional status of the body and has been studied primarily as a new factor involved in lipid metabolism. ANGPTL8 is mainly involved in the regulation of triglyceride levels by affecting LPL activity [13, 21, 22].

**Evidence of ANGPTL8 regulating lipid metabolism**

Ren et al. discovered that during differentiation of mouse and human primary preadipocytes, the transcriptional levels of ANGPTL8 increased significantly with lipid droplet formation, along with changes of biomarkers of adipocyte differentiation, such as PPAR-γ, SCD1, FABP4, PNPLA2, etc [13]. Moreover, knocking down ANGPTL8 results in a decrease in lipid content in adipocytes [24]. TNF-α is a cytokine that inhibits adipogenesis and dedifferentiates adipocytes, and down-regulates the expression of biomarkers for adipose differentiation. Studies have shown that during the culture of precursor fat cells, the addition of TNF-α can significantly down-regulate the expression of ANGPTL8 [25]. In addition, ANGPTL8 is also involved in lipolysis [26]. Some drugs that promote lipolysis, such as db-cAMP, forskolin, and isoproterenol, can significantly reduce the transcription levels of ANGPTL8. Loss of ANGPTL8 leads to a decrease in free fatty acids (FFAs). Consistently, the expression of genes involved in lipolysis (ANGPTL4 and leptin) and FA oxidation-related genes (Cpt1a, Cpt1b, and Pgc-1a) increases after knockdown of ANGPTL8 [13]. By using adenoviral vectors to overexpress ANGPTL8 or directly treating with recombinant ANGPTL8 protein, Zhang et al. found that ANGPTL8 can inhibit LPL activity and reduce triglyceride clearance in mice, thereby increasing serum triglyceride levels [21]. These results indicate that ANGPTL8 may play an important role in regulating functions of fat cells and lipid metabolism through affecting LPL activity.

**Interaction between ANGPTL3 and ANGPTL8**

ANGPTL3 is a hepatogenic secreted protein which also participates in lipid metabolism by regulating LPL activity. But the inhibitory effect of ANGPTL3 on LPL activity is not so strong that it can only play a role when its concentration is much larger than its physiological one [27]. Consistently, Chi et al. found that by injecting ANGPTL3 adenovirus, triglyceride levels in plasma in wild-type mice can be increased. The elevated levels of triglycerides are positively correlated with the amounts of adenosine injected [28]. This indicates that ANGPTL3 indeed participates in the regulation of LPL activity in a dose-dependent manner.

Studies have shown that ANGPTL3 relies on ANGPTL8 to play a regulatory role in lipid metabolism. In the presence of ANGPTL8, the minimum amount of ANGPTL3 was only 2 times of physiological concentration to significantly inhibit LPL activity [28]. On the contrary, injection of ANGPTL3 adenovirus into ANGPTL8 knockout mice did not significantly affect levels of triglyceride. This suggests that the mechanism by which ANGPTL3 regulates LPL activity may require the participation of ANGPTL8. ANGPTL8 can bind to the N-terminal domain of ANGPTL3 protein and facilitates cleavage of ANGPTL3 by exposing its cleavage site or recruiting relevant proteases. After the N-terminal domain is cleaved, the remaining part of ANGPTL3 is still bound to ANGPTL8 to form complexes which regulate LPL activity. In addition, Chi et al. also suggested that ANGPTL3 and ANGPTL8 can form complexes at different stoichiometric ratios, and the formation of such complexes occurs intracellularly, rather than outside of the cells after their secretion [28]. In fact, ANGPTL8 is rarely secreted extracellularly after intracellular synthesis. Although ANGPTL3 and ANGPTL8 do not have a fixed stoichiometric ratio when forming a complex, the ratio for a complex to exert the maximal LPL inhibition is approximately 1:1 [29].

Haller’s group found that the C-terminus of ANGPTL8 protein has a short helix structure that covers its binding site, so ANGPTL8 alone cannot inhibit LPL activity (Fig. 1). When ANGPTL3 is present, this binding site will be opened and the inhibitory role of ANGPTL8 becomes effective. Therefore, in traditional opinion, ANGPTL3 plays a primary role in the ANGPTL3/ANGPTL8 complex [10]. However, an ANGPTL3/8 complex with a variant of ANGPTL3 that lacks LPL inhibitory activity still inhibits LPL activity.
These results suggested that the activity of ANGPTL3 is not required for activity of the ANGPTL8/ANGPTL3 complex and the major inhibitory activity may be provided by ANGPTL8 [30]. In addition, a specific ANGPTL8 antibody that targets the C-terminus of ANGPTL8 without affecting the formation of the ANGPTL3/ANGPTL8 complex was used. This antibody can inactivate ANGPTL8, thereby reversing the inhibitory effects of the complex on lipoprotein lipase [28]. Therefore, they believe that ANGPTL8 may exert a major inhibitory effect on LPL activity in the ANGPTL3/ANGPTL8 complex.

It is well accepted that compared to their respective inhibitory effects of ANGPTL3 and ANGPTL8, the ANGPTL3/ANGPTL8 complex has greater inhibitory effects on LPL activity.

**Interaction between ANGPTL4 and ANGPTL8**

ANGPTL4 is a member of the ANGPTL protein family with approximately 50 kD in size. In mice, ANGPTL4 is mainly expressed in white and brown adipose tissues [31, 32]. Studies have shown that the N-terminal helix domain of ANGPTL4 can bind to LPL and change it from active dimers to form inactive monomers [33]. In addition, ANGPTL4 can also reduce the secretion of LPL in adipocytes [34-36].

Previous studies have suggested that ANGPTL4 exert its function in a homogeneous dimer linked by disulfide bonds [37]. Recent studies by Mysling’s group have shown that, similar to ANGPTL3, ANGPTL4 can also form a complex with ANGPTL8 (Fig. 2). However, the activity of ANGPTL4/8 complex to inhibit LPL is lower compared to the activity of ANGPTL4 alone. This seems to indicate that ANGPTL8 is a natural inhibitor of ANGPTL4. When the stoichiometric ratio is about 1:1, ANGPTL8 has the most obvious inhibitory effects on ANGPTL4, and this ratio is close to the physiological ratio of these two proteins in the human body [29].
The Differential Regulation of ANGPTL Proteins under Different Nutritional and Metabolic Status

The expression levels of ANGPTLs are mainly regulated by the nutritional status of the body [21] (Fig. 3). In the case of starvation, the expression of ANGPTL3 and 8 decreases, and re-feeding leads to an increase in expressions of ANGPTL3 and 8 [23]. Conversely, the expression of ANGPTL4 in adipose tissue increases when fast, and the expression decreases after feeding [38].

Starvation status

Under starvation, the expression of ANGPTL8 decreased by nearly 70% in WAT [21], and the expression of ANGPTL8 in BAT and liver was also significantly reduced [39]. Under starvation, circulating ANGPTL8/ANGPTL3 complex reduces [10], which increases LPL activity in peripheral tissues and in turn increases the uptake of VLDL by skeletal muscles and the heart. These processes ensure the energy supply to maintain the physiological functions of the body. On the contrary, fasting caused up-regulation of ANGPTL4 in WAT [21, 39]. The up-regulated ANGPTL4 reduces the uptake of circulating triglycerides by WAT through reducing LPL activity. In addition, up-regulated ANGPTL4 also increases the breakdown of white fat and secretion of fatty acids for energy supply [40].

Feeding status

Diet can increase the expression of ANGPTL8 in liver, and also increase the amounts of ANGPTL3, ANGPTL8, and ANGPTL3/8 complex in circulation. Increased ANGPTL3/8 complex in circulation inhibits the activity of LPL, and therefore the amounts of skeletal muscles and heart to absorb VLDL are reduced [10]. Therefore, energy is stored after eating. Studies have shown that X receptor alpha (LXRα) in liver plays an important role in the up-regulation of ANGPTL8 induced by feeding [23]. The expression of ANGPTL4 in white adipose tissue was down-regulated in abdominally obese subjects [38]. The increased expression of ANGPTL8 may form more ANGPTL4/ANGPTL8 complexes with ANGPTL4, thus reducing the inhibitory activity of the complexes [29]. Therefore, LPL activity increased in white adipose tissue, and triglyceride uptake increased in white adipose tissue. All these processes increased the storage and accumulation of energy.

ANGPTL8 participates in appetite regulation

The study showed that the ANGPTL8 levels in plasma in anorexia patients were higher than those in the normal control group [41]. Central or peripheral ANGPTL8 intervention significantly reduced food intake in mice. The hypothalamus has long been considered as a control center for regulating energy balance. ANGPTL8 is widely expressed in appetite-related nuclei, including paraventricular nucleus (PVN), dorsomedial hypothalamus (DMH), ventromedial hypothalamus, and hypothalamic arcuate nucleus (ARC) [42]. It has been indicated that hunger and satiety signals regulate the levels of ANGPTL8 and LPL is highly expressed in the brain. The information suggests that ANGPTL8 regulates the activity of LPL in brain, and ultimately regulates feeding behavior through hypothalamus (Fig. 4).

Cold stimulation

In response to cold stimulation, brown adipose tissue
produces heat. The expression of ANGPTL8 in BAT was up-regulated and ANGPTL4 was down-regulated at cold stimulation (4°C, 4 hours) [43]. In wild-type mice, cold stimulation can increase LPL-mediated degradation of triglyceride-rich lipoproteins (TRLs) in BAT to produce fatty acids for tremor heat production. The expression of ANGPTL8 increased when WAT is turned into BAT [44]. In addition, long-term ANGPTL8 exposure can increase the expression of BAT proteins such as UCP1, TMEM26, TBX1, and b3-AR during differentiation of hASC in vitro. These results suggest that ANGPTL8 may be involved in the regulation of lipid browning.

**ANGPTL8 and Metabolic Diseases**

Studies have shown that ANGPTL8 participates in lipid metabolism. It was reported that in diabetic patients, ANGPTL8 levels were significantly positively correlated with triglyceride (TG) and LDL-C levels, and negatively correlated with HDL-C levels [45, 46]. Javier et al. found that the circulating ANGPTL8 levels were significantly lower in patients with dyslipidemia characterized by low HDL-C or high TG levels [47]. A cross-sectional study of Chinese population showed that circulating levels of ANGPTL8 in full-length were significantly increased in patients with dyslipidemia compared with those without dyslipidemia. The circulating levels of ANGPTL8 in full-length were positively correlated with serum non-HDL-C, TG and TC levels, but negatively correlated with HDL-C levels [48]. The discrepancy among these results may be due to different blood glucose levels and diverse types of kits used. In addition, non-HDL-C is considered to be a strong predictor of the total number of atherogenic lipoprotein particles [49], indicating the total cholesterol of apolipoprotein B containing lipoproteins, including TG rich lipoproteins (very low density lipoproteins, medium density lipoproteins, and residual lipoproteins) [50].

In addition to lipid metabolism, ANGPTL8 may play a role in glucose metabolism by improving insulin resistance [19]. Under physiological status, the knockout of ANGPTL8 had no significant effects on glucose metabolism in mice [12]. In HEPG2 cells with insulin resistance, ANGPTL8 can increase glycogen synthesis and inhibit hepatic gluconeogenesis. Recently, ANGPTL8 has been widely investigated for its ability to promote the proliferation of β cells, but the results were eventually disagreed by several other paper [12, 51-54]. Although ANGPTL8 is not involved in the regulation of β cell proliferation, its correlation with endocrine and metabolic diseases, such as diabetes mellitus, has attracted much attention. Espes et al. observed an increase of circulating ANGPTL8 in patients with long-term type 1 diabetes mellitus [55]. Fu et al. also observed an increase in ANGPTL8 level in type 2 diabetic patients [56]. Our previous studies also showed that circulating ANGPTL8 levels in newly diagnosed type 2 diabetic patients increased by about two times [57]. Generally speaking, many studies have shown that levels of ANGPTL8 change in polycystic ovary syndrome (PCOS) [58, 59], metabolic syndrome [60, 61], non-alcoholic fatty liver [62, 63], hypothyroidism [64], Graves’ disease [65], and coronary heart disease [66].

**Conclusion**

ANGPTL3, ANGPTL4, and ANGPTL8 can all inhibit the activity of LPL by affecting the stability of LPL dimers. But their inhibition capability and tissue specificity are different and ANGPTL4 responds to nutrients opposingly compared to the other two. Generally speaking, ANGPTL8 is mainly expressed in liver and adipose tissues, ANGPTL3 is mainly expressed in liver, and ANGPTL4 is mainly expressed in adipose tissues. Feeding inhibits LPL activity in heart and skeletal muscles and causes WAT storage of circulating triglycerides through activating ANGPTL8/ANGPTL3 pathway. On the contrary, fasting inhibits LPL activity in WAT and induces oxidation of triglyceride in heart and skeletal muscles through activating ANGPTL4. In sum, this review discusses the interaction among ANGPTL3, 4, 8 proteins, and summarizes the relationship between the expression of these proteins and lipid metabolism under different nutritional and metabolic status.

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**Disclosure**

The authors have no conflicts of interest to declare.
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