Brief communication

MOLECULAR METABOLISM

# Differential effects of angiopoietin-like 4 in brain and muscle on regulation of lipoprotein lipase activity

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## ABSTRACT

**Objective:** Lipoprotein lipase (LPL) is a key regulator of circulating triglyceride rich lipoprotein hydrolysis. In brain LPL regulates appetite and energy expenditure. Angiopoietin-like 4 (Angptl4) is a secreted protein that inhibits LPL activity and, thereby, triglyceride metabolism, but the impact of Angptl4 on central lipid metabolism is unknown.

**Methods:** We induced type 1 diabetes by streptozotocin (STZ) in whole-body Angptl4 knockout mice (*Angptl4*$^{-/-}$) and their wildtype littermates to study the role of Angptl4 in central lipid metabolism.

**Results:** In type 1 (streptozotocin, STZ) and type 2 (ob/ob) diabetic mice, there is a ~2-fold increase of Angptl4 in the hypothalamus and skeletal muscle. Intracerebroventricular insulin injection into STZ mice at levels which have no effect on plasma glucose restores Angptl4 expression in hypothalamus. Isolation of cells from the brain reveals that Angptl4 is produced in glia, whereas LPL is present in both glia and neurons. Consistent with the *in vivo* experiment, *in vitro* insulin treatment of glial cells causes a 50% reduction of Angptl4 and significantly increases LPL activity with no change in LPL expression. In *Angptl4*$^{-/-}$ mice, LPL activity in skeletal muscle is increased 3-fold, and this is further increased by STZ-induced diabetes. By contrast, *Angptl4*$^{-/-}$ mice show no significant difference in LPL activity in hypothalamus or brain independent of diabetic and nutritional status.

**Conclusion:** Thus, Angptl4 in brain is produced in glia and regulated by insulin. However, in contrast to the periphery, central Angptl4 does not regulate LPL activity, but appears to participate in the metabolic crosstalk between glia and neurons.



**Keywords** Angptl4; Lipid metabolism; Lipoprotein lipase

## 1. INTRODUCTION

Over the past decade there has been increasing evidence indicating an essential role of the central nervous system in the regulation of energy homeostasis, development of obesity and glucose intolerance [reviewed in Ref. [1]]. Free fatty acids (FFA), and especially long chain fatty acids, have been shown to inhibit food intake and hepatic glucose production [2]. The major pools of FFA within the circulation are contained in triglyceride-rich lipoproteins and or bound to albumin.

Lipoprotein lipase (LPL) is the key enzyme that controls the hydrolysis of triglyceride-rich lipoproteins into FFA, which can then be used for storage or consumption in peripheral tissues. Activity of LPL is governed by a number of different mechanisms, most of which act at the posttranscriptional and posttranslational levels [3]. Angiopoietin-like (Angptl) 4 is a known inhibitor of LPL activity. Angptl4 is primarily expressed in adipose tissue and liver, induced by fasting and secreted into the circulation [4], where it prevents LPL dimerization and thereby preventing LPL activation [5–8]. Studies using genetically modified mouse models of Angptl4 confirm its key role in triglyceride metabolism. Thus, Angptl4 knockout mice have reduced plasma triglycerides, whereas Angptl4 overexpressing mice have increased triglyceride levels [9,10]. Central Angptl4 has been suggested as a modulator of food intake [11] and a protector of brain tissue from ischemia induced alterations [12]. However, its role in central lipid metabolism remains unknown.

The impact of triglyceride hydrolysis and FFAs in the central nervous system is still not completely understood, but inactivation of LPL in neurons of mice affects both food intake and energy expenditure, resulting in obesity [13]. This suggests that regulation of triglyceride metabolism in the brain is important in control of whole body metabolism. LPL is synthesized in various tissues including adipose tissue, skeletal muscle, heart lung, kidney and spread along the vascular endothelium [3]. In addition, LPL is present in most regions of the brain, particularly in areas with high levels of neuronal cell bodies, but to a lesser extent in areas rich in glial cells [14]. The function of LPL in the brain still remains elusive, but it has been implicated in at least two

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*Abbreviations:* AgRP, agouti-related protein; Angptl4, angiopoietin-like 4; ARC, arcuate nucleus; CART, cocaine-and-amphetamine-regulated transcript; CNS, central nervous system; FFA, free fatty acid; LPL, lipoprotein lipase; NPY, neuropeptide-Y; POMC, pro-opiomelanocortin; STZ, streptozotocin; TG, triglyceride

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different functions; the regulation of body weight and energy balance as well as cognition [14,15]. In the present study we have explored the regulation and role of Angptl4 in the brain in mouse models of diabetes and through the use of the Angptl4 knockout mice. Since LPL is known to affect whole body glucose homeostasis dependent on expression level in skeletal muscle [16,17], this tissue was included in the analysis to compare differences between central and peripheral regulated lipid metabolism.

## 2. MATERIAL AND METHODS

### 2.1. Animals

C57Bl/6 and ob/ob (C57Bl/6 background) mice were obtained from Jackson laboratory (Bar Harbor, ME). *Angptl4*$^{-/-}$ mice (mixed genetic background; C57Bl/6, 129Sv) were provided by Jeffrey Gordon (Washington University) [18]. All mice used for experiments were male. For STZ induced diabetes, seven-week-old C57Bl/6 or 10–14-week-old *Angptl4*$^{-/-}$ and their wildtype littermates were treated with a single intraperitoneal (i.p.) injection (150 mg/kg bodyweight) of streptozotocin (STZ, Sigma). At day seven mice were sacrificed, and serum and tissues collected. All protocols were in accordance with NIH guidelines and approved by the Institutional Animal Care and Use Committees of the Joslin Diabetes Center and Brandeis University. Glucose levels were measured with Infinity glucose monitors (US Diagnostics), insulin (Chrystal Chem) and triglycerides (Abnova) were measured according to the manufacturers' protocols.

### 2.2. Insulin and Angptl4 intracerebroventricular (i.c.v.) administration

A 26-gauge guide cannula (Plastics One Inc., Roanoke, VA) was inserted into the right lateral cerebral ventricle of seven-week-old C57Bl/6 mice as described previously [19]. At day seven, the mice received a single i.p. injection of STZ to induce diabetes. Twelve days later the mice received three intracerebroventricular (i.c.v.) injections of insulin (3 mU in 2 μl) or 2 μl phosphate buffered saline (PBS) (9 A.M., 7 P.M., and 9 A.M. the following day) [19]. Food intake was measured immediately before the i.c.v. injection. Four hours after the last injection, blood glucose levels were measured, and hypothalami collected. Administration of human recombinant Angptl4 (Axxora, San Diego, CA) or saline was delivered (10 ng/h) using an osmotic pump (Durect Corp., Cupertino, CA) and a 26-gauge guide cannula (Plastics One, Roanoke, VA) located 1.0 mm posterior and 1.0 mm lateral from the bregma.

### 2.3. Primary culture glia and cortical neurons for insulin and glucose stimulation

Primary glial cells and neurons were isolated as previous described [19]. Glial cells were harvested 14 days after initial plating, while neurons were harvested 18 days after initial plating. For insulin stimulation, glial cells were serum deprived in media containing 0.1% BSA for 6 h before stimulated with 0, 10 or 100 nM insulin in 5.5 or 15 mM glucose for 3, 6 and/or 24 h.

### 2.4. Quantitative real-time PCR

RNA from mouse tissue and cell samples was extracted using RNeasy Mini Kit (Qiagen) and reverse-transcribed (1 μg) with high-capacity cDNA reverse transcription kit (Applied Biosystems). Real-time PCR was performed in ABI Prism 7900 HT sequence detection system (Applied Biosystems) using a final volume of 10 μl per reaction with SYBR Green PCR Master Mix (Applied Biosystems), 12.5 ng of cDNA and 300 nM sense and antisense primers. Analysis of TATA box-binding protein (TBP) expression was performed in parallel to normalize gene expression and analyzed by using the $2^{-\Delta\Delta Ct}$ method. Primer sequences are given in Supplementary Table 1.

### 2.5. LPL activity

Mice were either random fed or fasted overnight before sacrificed, and tissues were removed and snap frozen. Heparin-releasable LPL was assayed as described [20]. For skeletal muscle and cerebral cortex, 40–50 mg tissue was used to assess LPL activity, expressed in nmol FFA/min/g tissue, whereas only 10 mg tissue was used for hypothalamus. For glial cells, media was collected and cells were harvested in lysis buffer (50 mM Tris–HCl, pH 7.4, 150 mM NaCl, 0.25% deoxycholic acid, 1% NP-40, 1 mM EDTA) containing protease inhibitor mixture and phosphatase inhibitor cocktail 1 and 2 (Sigma). Protein concentration was determined using BCA method (Pierce), and LPL activity was expressed as nmol FFA/min/μg protein. For further details, see Supplementary materials.

### 2.6. Statistics

All results are expressed as mean ± SEM. Significance was established using the 2-tailed Student's *t* test and ANOVA when appropriate. Differences were considered significant at $p < 0.05$.

## 3. RESULTS

### 3.1. Regulation of hypothalamic Angptl4 in diabetes by insulin

In an attempt to determine how diabetes affects brain metabolism and hypothalamic function, we performed global gene expression on the hypothalamus from animal models of type 1 diabetes (streptozotocin, STZ) and type 2 diabetes/obesity (ob/ob) [19]. As expected, the STZ-induced diabetic mice were hyperglycemic, had low insulin levels and lost weight compared to controls, as opposed to the ob/ob mice, which were hyperglycemic, hyperinsulinemic and obese (Figure 1A–C). One of the most significantly upregulated genes in the hypothalami of both STZ diabetic mice and ob/ob mice compared to controls by microarray analysis was angiopoietin-like 4 (Angptl4). Angptl4 mRNA expression was confirmed by qPCR, which revealed 1.5–3-fold increases in these two models of diabetes, respectively (Figure 1D and F). LPL activity in skeletal muscle is known to be positively correlated with insulin sensitivity and negatively correlated with fasting insulin levels [21]. Indeed, a similar upregulation of Angptl4 was observed in skeletal muscle from the STZ and ob/ob models described above (Figure 1E), suggesting that Angptl4 might be regulated and act the same way centrally as in skeletal muscle. Importantly, in the STZ-diabetic mice, upregulation of Angptl4 in the hypothalamus could be normalized by direct i.c.v. injection of insulin at a dose (3 mU per mouse) which does not affect blood glucose levels (Figure 1F, [19]). Taken together, these data indicate that Angptl4 expression in the brain is highly dysregulated in diabetes and can be normalized by increasing brain insulin levels without normalizing blood glucose.

### 3.2. Differential expression of Angptl4 in neurons and glia

It is well known that there is nutritional crosstalk between neurons and glial cells, including transfer of lipid and other metabolites between these two cell types [22]. To determine which cells in the brain might make Angptl4, we isolated primary neurons and glial cells from wildtype mice (Supplementary Figure 1A and B). Interestingly, we found that Angptl4 was almost exclusively expressed in glial cells, with almost none detected in neurons (Figure 2A). By contrast, LPL mRNA was clearly expressed in both neurons and glial cells, although it was also slightly higher (~37%) in glia. Six hours of insulin treatment *in vitro* significantly downregulated expression of Angptl4 in glial cells,

 