

# Roles of activin receptor-like kinase 7 signaling and its target, peroxisome proliferator-activated receptor $\gamma$ , in lean and obese adipocytes

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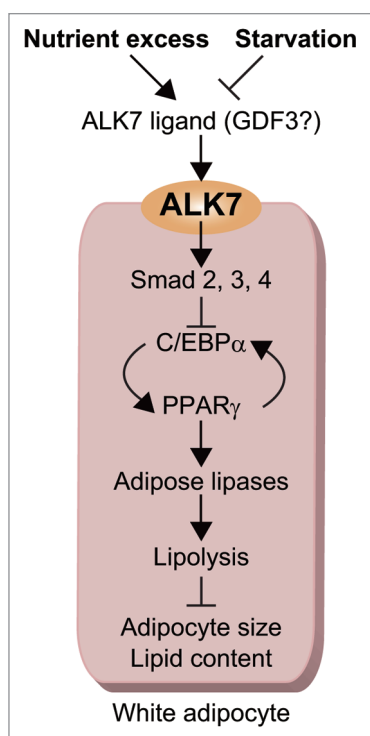
We recently discovered a novel signaling pathway involving activin receptor-like kinase 7 (ALK7), one of the type I transforming growth factor- $\beta$  receptors. ALK7 and activated Smads 2, 3, and 4 inhibit the master regulators of adipogenesis, CCAAT/enhancer-binding protein  $\alpha$  (C/EBP $\alpha$ ) and peroxisome proliferator-activated receptor  $\gamma$  (PPAR $\gamma$ ) specifically in differentiated adipocytes, but surprisingly increase both the adipocyte size and lipid content by suppressing lipolysis. Here, we show that, although both transcription factors are suppressed by ALK7 in either the obese or lean state, PPAR $\gamma$ , but not C/EBP $\alpha$ , is further suppressed under obesity through an ALK7-independent pathway. As a result, PPAR $\gamma$  and adipose lipolytic activities are severely downregulated in obesity. Reactivation of PPAR $\gamma$  by ALK7 inactivation leads to downregulation of inflammatory adipocytokines and upregulation of adiponectin. We propose that PPAR $\gamma$  promotes lipid turnover and remodeling by stimulating both triglyceride synthesis and breakdown in differentiated adipocytes. Finally, we discuss the physiological and evolutionary roles of the ALK7-signaling pathway and consider it as a potential target of therapy for obesity.

Through positional cloning of *Nidd5*, the previously identified quantitative trait locus for body weight,<sup>1</sup> we recently demonstrated a nonsense mutation of the *Acvr1c* gene encoding activin receptor-like kinase 7 (ALK7) in the genome of BALB mice.<sup>2</sup> The ALK7 dysfunction decreases adiposity by increasing lipolysis in mature adipocytes. Conversely, ALK7 activation strongly depresses the expressions of adipose triglyceride lipase (ATGL) and hormone-sensitive lipase (HSL), two major lipases in white adipose tissue (WAT).<sup>3</sup> Mechanistically, ALK7 promotes phosphorylation and nuclear entry of Smads 2, 3, and 4, which then bind CCAAT/enhancer-binding protein  $\alpha$  (C/EBP $\alpha$ ) to inhibit its transactivation function. This interrupts the positive feedback loop between C/EBP $\alpha$  and peroxisome proliferator-activated receptor  $\gamma$  (PPAR $\gamma$ ),<sup>4</sup> and markedly decreases the expression of those transcription factors themselves and that of adipose lipases (Fig. 1). One of the surprising findings in this study is that PPAR $\gamma$  and C/EBP $\alpha$  induce net lipolysis and decrease the size of mature adipocytes, although those transcription factors are essential for adipogenesis and thus are generally considered to be adipogenic. However, it should be noted that ALK7 is expressed only in differentiated adipocytes and that this novel signaling pathway does not function during adipocyte differentiation, although TGF- $\beta$  has been suggested to inhibit adipocyte differentiation by the similar mechanism.<sup>5,6</sup> Furthermore, the upregulation of PPAR $\gamma$  and C/EBP $\alpha$  in ALK7-deficient adipocytes not only leads to triglyceride (TG) breakdown but also to TG synthesis, although

the net lipid storage is decreased.<sup>2</sup> Based on these findings, we propose that PPAR $\gamma$  and C/EBP $\alpha$  play a pivotal role in the lipid remodeling of mature adipocytes by stimulating lipid turnover.

According to this scenario, the activation of those transcription factors in obese adipocytes may have a beneficial effect by reducing their cell size. To test this hypothesis, we chose several lean and obese mice with and without the ALK7 mutation (Fig. 2A) and examined the expression levels of adipose-specific PPAR $\gamma$ 2 and C/EBP $\alpha$  in their adipocytes (Fig. 2B). We first confirmed the previous findings<sup>2</sup> that both transcription factors were markedly upregulated in adipocytes from T.B-*Nidd5/3* congenic mice, which incorporate the defined genome region of BALB mice containing the ALK7 mutation in the genetic background of Tsumura, Suzuki, Obese Diabetes (TSOD) mice,<sup>7</sup> compared with those in adipocytes from parental TSOD mice possessing wild-type ALK7. The higher expression levels of PPAR $\gamma$ 2 and C/EBP $\alpha$  by ALK7-deficiency were also detected in lean mice, namely between ALK7-mutated BALB mice and ALK7-intact C57BL/6 or C3H/He mice (we confirmed that the latter two strains do not have the *Acvr1c* gene mutation found in BALB mice). This finding suggests that the ALK7 signal is also active under the lean state. Notably, the expression level of PPAR $\gamma$ 2, but not that of C/EBP $\alpha$ , was suppressed by the obese state itself irrespective of the presence or the absence of functional ALK7 (comparison of the PPAR $\gamma$ 2 expression levels in ALK7-intact mice between obese TSOD and lean C57BL/6 or C3H/He mice,

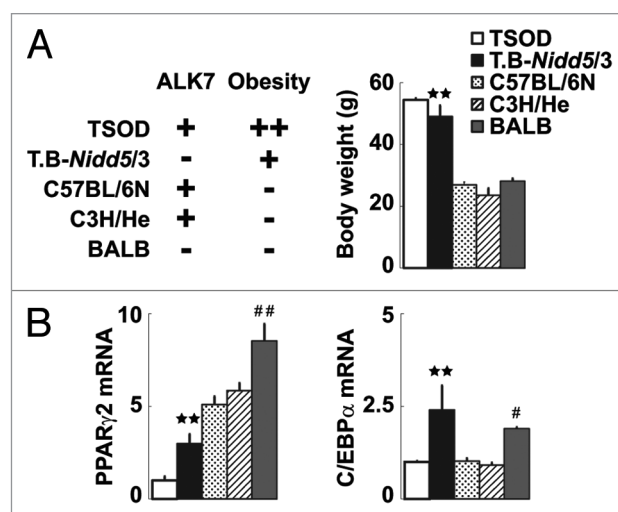
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**Figure 1.** The scheme of ALK7 signaling and its function in differentiated adipocytes. Although the natural ligand of ALK7 receptor has yet to be established, growth/differentiation factor 3 (GDF3) has been suggested as a candidate.<sup>22</sup> We propose that ALK7 signaling is transiently activated under nutrient excess after food intake to store TG in adipocytes, and is chronically activated under obesity. Activated ALK7 phosphorylates Smad2 and/or 3, which in turn binds C/EBP $\alpha$  with Smad4 in the nucleus and inhibits the expression of PPAR $\gamma$  and C/EBP $\alpha$  itself.<sup>2</sup> PPAR $\gamma$  and C/EBP $\alpha$  positively regulate the expression of adipose lipases such as ATGL and HSL.

$P < 0.01$ ; and in ALK7-mutated mice between obese T.B-*Nidd5/3* and lean BALB mice,  $P < 0.01$ ), although each strain has a different genetic background except between TSOD and T.B-*Nidd5/3* mice. Resultantly, PPAR $\gamma$  is most severely suppressed in adipocytes of TSOD mice. These findings indicate that PPAR $\gamma$  is downregulated under obesity by both ALK7-dependent and -independent pathways.

We then compared the expression levels of TG lipases in adipocytes among those five kinds of mice. We confirmed that the transcript levels of ATGL and HSL were much higher in ALK7-deficient adipocytes from the T.B-*Nidd5/3* mice compared with those from the TSOD mice, as reported previously.<sup>2</sup> The increased expression by ALK7 deficiency was also found in lean mice among BALB, C57BL/6, and C3H/He mice (Fig. 3A). We further examined the expression levels of other lipases that possibly function in adipocytes. Triacylglycerol hydrolase (TGH),<sup>8</sup> and the related TGH-2,<sup>9</sup> are expressed in adipocytes and are suggested to be involved in the lipolysis of stored TG. In fact, TGH-deficient mice have recently been shown to exhibit a 70% decrease in total in vitro hydrolase activity in WAT homogenates and a 70% increase in WAT weight,<sup>10</sup> suggesting that TGH plays a significant role in lipolysis. The transcript levels of TGH and

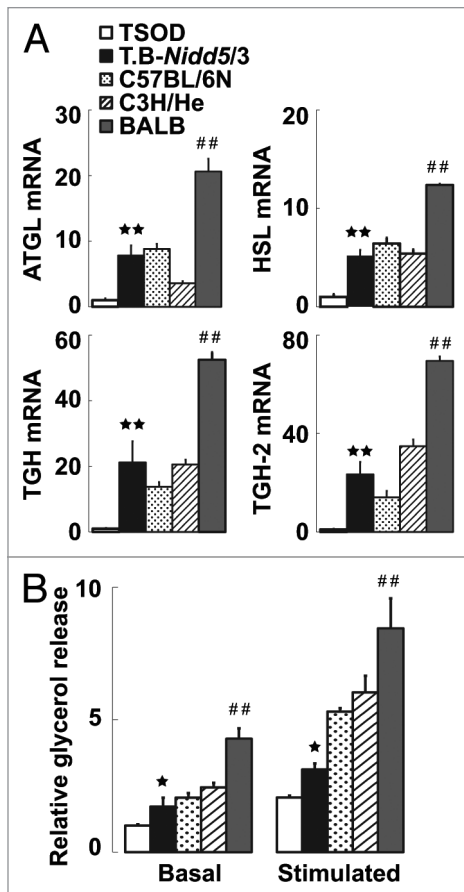


**Figure 2.** ALK7 downregulates PPAR $\gamma$  and C/EBP $\alpha$  in adipocytes of obese and lean mice. (A) Body weight of 10-week-old male TSOD, T.B-*Nidd5/3*, C57BL/6N, C3H/He, and BALB mice containing either functional (+) or nonfunctional (-) ALK7. Data are means  $\pm$  SD ( $n = 3\text{--}6$  per each group). (B) PPAR $\gamma$ 2 and C/EBP $\alpha$  mRNA expression in adipocytes isolated from 10-week-old male mice. Quantitative PCR analyses were performed as described previously.<sup>2</sup> Data are means  $\pm$  SD ( $n = 3$  per each group). \*\* $P < 0.01$  vs. TSOD mice, # $P < 0.05$ , ## $P < 0.01$  vs. C57BL/6N and C3H/He mice; one-way ANOVA.

TGH-2 were again much higher in ALK7-deficient T.B-*Nidd5/3* adipocytes compared with TSOD adipocytes, and in ALK7-deficient BALB adipocytes compared with C57BL/6N or C3H/He adipocytes (Fig. 3A). Taken together, these findings indicate that loss of functional ALK7 leads to marked elevation of universal adipose lipases under both obese and lean states.

Consistent with the expression levels of adipose lipases in the present study (Fig. 3A) and in previous reports,<sup>2</sup> glycerol release from adipocytes of ALK7-deficient T.B-*Nidd5/3* mice was significantly increased in both the basal and isoproterenol-stimulated states compared with that of TSOD mice (Fig. 3B). The elevated lipolysis by ALK7 deficiency was also found under the lean state between BALB mice and C57BL/6 or C3H/He mice. Consistent with the expression levels of TG lipases (Fig. 3A), the lipolytic activity in lean mice was higher than that in obese mice irrespective of the presence or absence of functional ALK7 (Fig. 3B). Overall, the lipolytic activities were highly correlated with the expression levels of PPAR $\gamma$ 2 (Fig. 2B), suggesting that PPAR $\gamma$ 2 principally dominates adipose lipolytic activities. These findings are consistent with previous findings that PPAR $\gamma$  agonism increases expression of ATGL and HSL in adipose tissues and 3T3-L1 adipocytes.<sup>11-13</sup>

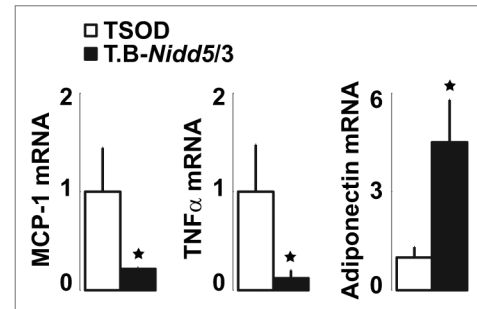
The notable finding in the present study is that PPAR $\gamma$  and PPAR $\gamma$ -dependent lipolysis are severely downregulated in obesity. Impaired adipose lipolysis, as well as decreased expression of HSL and ATGL, has actually been observed in obese human subjects.<sup>14-17</sup> Therefore, dysfunction of PPAR $\gamma$  in adipocytes may well be a hallmark of obesity that leads to decreased mobilization of TG and to increased fat accumulation. Such locally inert lipid droplets might cause chronic



**Figure 3.** Loss of functional ALK7 leads to increased lipolysis in mouse adipocytes. (A) ATGL, HSL, TGH, and TGH-2 mRNA expression in adipocytes from 10-week-old male TSOD, T.B-*Nidd5/3*, C57BL/6N, C3H/He, and BALB mice. (B) Basal and isoproterenol-stimulated glycerol release from adipocytes of 10-week-old male mice. Data are means  $\pm$  SD ( $n = 3\sim 5$  per each group). \* $P < 0.05$ , \*\* $P < 0.01$  vs. TSOD mice; ## $P < 0.01$  vs. C57BL/6N and C3H/He mice; one-way ANOVA.

inflammation and insulin resistance on the whole-body level, as proposed previously.<sup>18</sup> In fact, inflammatory cytokines such as monocyte chemoattractant protein-1 and tumor necrosis factor- $\alpha$ <sup>19</sup> were downregulated in adipocytes from ALK7-deficient T.B-*Nidd5/3* mice compared with those from TSOD mice (Fig. 4). By contrast, insulin-sensitizing, anti-inflammatory adiponectin<sup>20</sup> was upregulated in ALK7-deficient adipocytes. These findings suggest that the increased lipid turnover ameliorates the metabolic and/or inflammatory dysfunction associated with obesity.

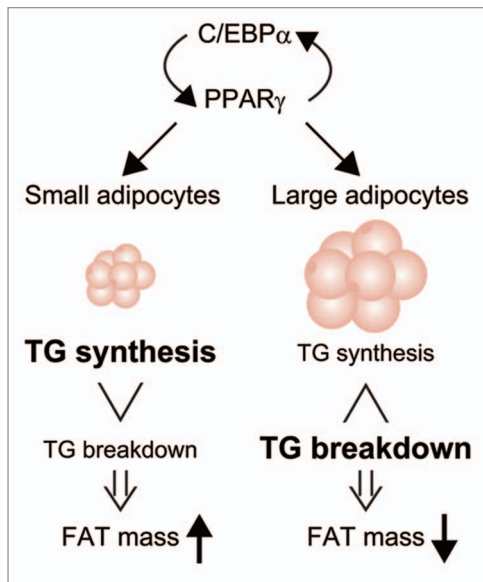
As stated above and reported previously,<sup>2</sup> upregulation of PPAR $\gamma$  and C/EBP $\alpha$  due to ALK7 deficiency promotes lipolysis and decreases fat mass in differentiated adipocytes. By contrast, those transcription factors appear to have the opposite effect during adipocyte differentiation by promoting lipid storage. However, it is important to emphasize that the net effects could depend on the amount of TG in adipocytes. While the lipid storage rate mainly relies on the uptake of fatty acids (FAs) derived from exogenous sources such as food, the lipid removal rate clearly depends on the amount of lipids already stored in adipocytes. Thus, although the function of PPAR $\gamma$  and C/EBP $\alpha$



**Figure 4.** Expression levels of monocyte chemoattractant protein-1 (MCP-1), tumor necrosis factor- $\alpha$  (TNF $\alpha$ ), and adiponectin in adipocytes isolated from 10-week-old male TSOD and T.B-*Nidd5/3* mice. Data are means  $\pm$  SD ( $n = 3$  per each group). \* $P < 0.05$  vs. TSOD mice.

may always be to stimulate both TG synthesis and breakdown, their activation leads to fat accumulation when the adipocyte size is small during differentiation, whereas it results in fat decrease when it is large, particularly in an obese state, depending on the relative contribution of lipolysis (Fig. 5). As such, PPAR $\gamma$  and C/EBP $\alpha$  could maintain the physiological size and lipid content of adipocytes, which may be their major function in differentiated adipocytes. This idea may explain discrepant findings about the effects of PPAR $\gamma$  and its agonists on the cell size and intracellular TG contents in cultured 3T3-L1 adipocytes,<sup>2,21</sup> which could contain varying amounts of lipids depending on the experimental protocol employed. Furthermore, it may also account for the differential phenotypes between ALK7-deficient obese and lean mice: the obese mice show decreases in body weight and fat mass,<sup>2</sup> whereas the lean mice do not.<sup>22</sup>

This brings into question the physiological function of ALK7. Because the ALK7 signal functions in both lean and obese states as indicated above, we speculate that the ALK7 signal is transiently activated after food intake to store excess nutrients as fat in adipocytes by suppressing adipose lipases (Fig. 1). This lipid storage function could reasonably be envisioned as a defense against starvation that confers an evolutionary advantage. However, given the food excess in modern developed countries, sustained activation of the ALK7 signal might impair optimal lipid storage and mobilization. Therefore, the inhibition of the ALK7 signal may be a potential target of therapy for obesity. If PPAR $\gamma$  is selectively activated in mature adipocytes by inhibiting the ALK7 signal, it could improve insulin sensitivity more efficiently than systemically administered PPAR $\gamma$  agonists such as thiazolidinedione, because weight gain mediated via brain PPAR $\gamma$ <sup>23,24</sup> should not occur. Although the increased lipolysis induced by ALK7 inhibition may elevate circulating FA levels and cause ectopic fat accumulation and/or insulin resistance,<sup>25</sup> it must be understood that this depends on the context of the nutrient state. In fact, it has been shown that lean ALK7-deficient mice exhibit hepatic steatosis and insulin resistance as they age,<sup>26</sup> whereas obese ALK7-deficient mice exhibit no elevation of serum FAs or hepatic TG content but show increased insulin sensitivity and glucose tolerance.<sup>2</sup> In summary, we emphasize the importance of PPAR $\gamma$  reactivation in obesity and propose that inhibition of the ALK7 signal represents a promising mechanism and



**Figure 5.** A model for the action of PPAR $\gamma$  and C/EBP $\alpha$  in differentiating (small) and differentiated (large) adipocytes. Although PPAR $\gamma$  and C/EBP $\alpha$  act as adipogenic transcription factors during adipocyte differentiation, they appear to promote net lipolysis to decrease the cell size and fat mass in differentiated adipocytes.<sup>2</sup> Change in the size of the fat mass is determined by the balance between the amount of lipid storage by adipocytes and the rate of lipid removal from the body. In contrast to the lipid storage that depends on the uptake of nutrients such as FAs into adipocytes, the rate of lipid removal through lipolysis is proportional to the total fat mass as well as the activities of lipases. We thus propose that those transcription factors promote net TG synthesis when the size of adipocytes is small during differentiation because of the low rate of lipolysis, whereas they promote net TG breakdown when it is large, particularly in obesity, due to the higher lipolytic rate. PPAR $\gamma$  downregulated under obesity in differentiated adipocytes by the ALK7-dependent pathway and by other unknown mechanisms (see text and Fig. 2) is considered a potential target of medical intervention for obesity.

potential target for therapeutic intervention in obesity and associated metabolic dysfunction by controlling the adipose master regulators, PPAR $\gamma$  and C/EBP $\alpha$ , specifically in differentiated adipocytes.

#### Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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