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REVIEW

Mechanism of Compensatory Growth with Changing Levels of Dietary Lysine from Deficient to Sufficient in Pigs

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Abstract

This review describes our studies on compensatory growth, specifically the growth that occurs after dietary lysine deficiency is changed to sufficiency. We found that dietary lysine sufficiency induced compensatory growth in pigs after dietary lysine deficiency. We also showed that compensatory growth of pigs induced by dietary lysine sufficiency was partly attributed to greater N retention. In a rat model, both suppression of proteolysis and increased protein synthesis in skeletal muscle contributed to their compensatory growth with lysine sufficiency. Finally, our in vitro studies with cultured skeletal muscle cells revealed that the compensatory growth in these cells was induced by increased lysine levels in combination with the modulation of insulin-like growth factor-I and glucocorticoid levels. This suggested that compensatory growth in pigs and rats with lysine sufficiency was due to both increased serum lysine levels and changes in the levels of hormones involved in protein synthesis and degradation.

Discipline: Animal industry
Additional key words: C2C12, pig, rat

Introduction

At the beginning of last century, Osborne & Mendel (1916) reported that when animals with retarded growth due to undernutrition received a normal diet, their subsequent growth rate was higher than normal for their chronological age. This phenomenon was termed “compensatory growth” by Bohman (1955). Figure 1 is a schematic representation of the growth paths of normal and restricted-refed animals.

Numerous studies have since examined compensatory growth in growing pigs that have been subjected to restricted feed intake or protein restriction (Chaosap et al. 2011, Fabian et al. 2002, Heyer & Lebret 2007, Oksbjerg et al. 2002). In these studies, compensatory growth was explored as a means of improving carcass meat quality, nutrient utility, and nitrogen excretion and thus improving pork production efficiency. These studies indicate that sufficiency in dietary protein and amino acid contributes to triggering compensatory growth in pigs. However, compensatory growth was only observed in some studies (Chaosap et al. 2011, Martínez-Ramírez et al. 2009, Martinez-Ramirez et al. 2008, O’Connell et al. 2006); in other studies, compensatory growth was incomplete or absent (Chiba et al. 1999, Pond & Mersmann 1990, Skiba et al. 2001, Yang et al. 2008a). Therefore, how compensatory growth can be induced and the underlying mechanisms remain to be elucidated.

The roles played by specific amino acids in compensatory growth also remain to be determined because in the diet used for the previous studies on the compensatory growth of restricted-refed pigs, the levels of multiple amino acids fed to pigs differed for each diet tested. Our interest was in single amino acid, lysine, after our laboratory had generated

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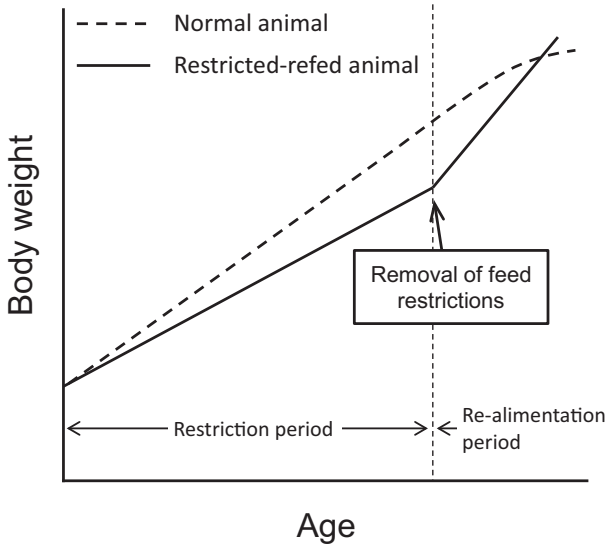

Fig. 1. Schematic representation of the growth paths of normal and restricted-refed animals

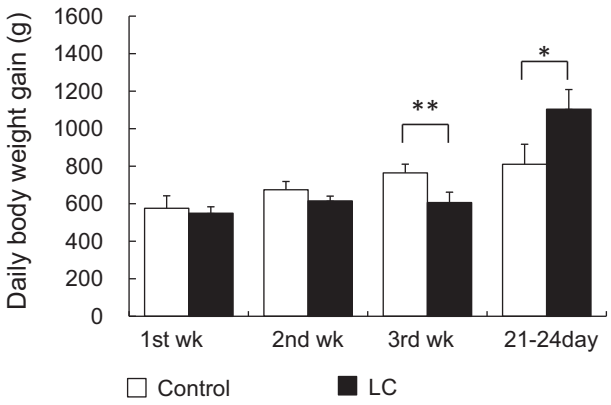

Fig. 2. Effect of lysine sufficiency on (A) average daily body weight gain in pigs of control and low-lysine subsequent control diet (LC) groups. Values are means ± SE. * P < 0.05 compared with the first 21 days. n=6, At each term, points with different superscript letters differ significantly (P < 0.05). Data from Ishida et al. (2012)

data concerning lysine deficiency. As emerged with protein or multiple amino acid deficiencies, pigs fed low-lysine diets grow more slowly and less efficiently than those fed a control diet (Katsumata et al. 2002). This observation lead us to hypothesize that changing the lysine dietary levels from deficiency to sufficiency would induce compensatory growth in growing pigs. Indeed, there is some evidence that dietary lysine sufficiency may induce compensatory growth in protein-restricted-refed pigs because lysine is the first major limiting essential amino acid in the most pig diets. However, in these studies, lysine was simply used an indicator of protein restriction, which means that sufficiency in multiple amino acids, not just lysine, may have been involved in compensatory growth (Fabian et al. 2002, Yang et al. 2008b). This study design were not appropriate for elucidating the mechanism of compensatory growth. The reproducible compensatory growth induced by the single amino acid, lysine, may help investigate the mechanism of compensatory growth.

In this paper, we describe the results of our first study on compensatory growth induced by lysine in pigs (Ishida et al. 2012). We then describe our studies using a rat model (Ishida et al. 2011) and an in vitro model with cultured skeletal muscle cells (Ishida et al. 2013) to elucidate the mechanisms of compensatory growth after restriction-realimentation with lysine alone.

Nitrogen balance during compensatory growth with changing dietary lysine levels from deficient to sufficient in growing pigs

We first tested whether changing dietary lysine levels from deficient to sufficient would induce compensatory growth in growing pigs, as measured by body weight. We prepared two diets: a control diet with 1.15% lysine and a low-lysine diet in with 0.73% lysine (Ishida et al. 2012). The diets were iso-protein and iso-energetic, meaning both diets had the same concentrations of all essential amino acids except lysine and met the nutritional requirements (NRC, 1998). The experiments began when the pigs were six weeks of age. The control pigs were fed the control diet continuously throughout the 24 days of the experimental period. The low-lysine subsequent control diet (LC) pigs were fed the low-lysine diet until day 21 of the experiment and then the control diet for three days until the end of the experiment. Accordingly, the LC pigs experienced a shift in the nutritional status of lysine from deficiency to sufficiency. As hypothesized, the dietary lysine content markedly affected the performance of the pigs (Fig. 2): in the third week, pigs fed a low-lysine diet had a lower growth rate and feed efficiency than the control pigs but after they returned to a normal diet, they exhibited compensatory growth. On day 21, the pigs fed a low-lysine diet had significantly lower serum IGF-I levels (303 ng/mL) than the normal pigs (424 ng/mL; P<0.05). On day 24 (i.e. 3 days after changing the diet), the two groups did not differ in terms of serum IGF-I concentrations. This led us to hypothesize that the initial decrease in growth rate in the lysine-restricted pigs and the subsequent enhancement in growth after lysine-sufficiency was involved in protein accumulation because IGF-I play a crucial role in protein metabolism.

To obtain further insights into the composition of body weight gain during compensatory growth of the pigs, we focused on the nitrogen (N) balance in pigs exhibiting com-

pensatory growth with dietary lysine sufficiency. From day 21 to 24, the amount of N retention in the LC pigs per metabolic body weight (2.51 g/ $BW^{0.75}$ kg) exceeded that of the control pigs (2.05 g/ $BW^{0.75}$ kg; $P<0.05$). The efficiency of N retention (retention/intake) of the pigs was also lower in the pigs fed the low-lysine diet from day 19 to 21, whereas after changing the diet, no differences in N retention efficiencies were observed between the LC and control pigs. Accrodingly, the increased N retention was related to compensatory growth with dietary lysine sufficiency.

Muscle protein metabolism during compensatory growth induced by lysine sufficiency in rats

Our previous study revealed that, the compensatory growth in pigs was at least partly attributable to higher nitrogen retention. The amount of protein accumulation depends on the balance between protein synthesis and protein degradation. However, it was unclear whether lysine sufficiency caused protein synthesis to increase and/or protein degradation to decrease. To investigate muscle protein metabolism with compensatory growth induced immediately after dietary lysine sufficiency, a model for rats was established because protein synthesis and degradation in these animals can be estimated by measuring urine 3-methylhistidine concentrations (Nishizawa et al. 1977, Wassner et al. 1980). We prepared two diets: a control diet with 1.30% lysine (which meets the requirements of growing rats) and a low-lysine diet with 0.46% lysine. The control rats were continuously fed with the control diet throughout the 21-day experimental period. The LC rats were fed the low-lysine diet for the first 14 days, after which they were fed the control diet until the end of the experiment.

The changing dietary levels of a single essential amino acid from deficient to sufficient induces compensatory growth in growing rats. The gastrocnemius muscle weight per body weight of the LC group was higher than that of the control rats on the day 21 (i.e. 7 days after changing the diet). In rats, the skeletal muscle ratio per body weight is high and skeletal muscle rapidly accumulates protein during the growing phase (Bates & Millward 1981). Thus, increased skeletal muscle growth may contribute markedly to the compensatory growth observed in LC rats. Indeed, increased protein synthesis of skeletal muscle continuously contributed to the growth of rats from day 14 to 21 (i.e. 1 to 7 days after changing the diet) while suppression of proteolysis contributed to the growth during the first 3 days after the lysine sufficiency (Fig. 3) Thus, suppression of proteolysis and increased protein synthesis both contributed to the enhanced growth rate after lysine sufficiency, although there were differences in the timing of these contributions. The values of serum concentrations of lysine in rats fed the lysine-deficient diet promptly returned to the control values

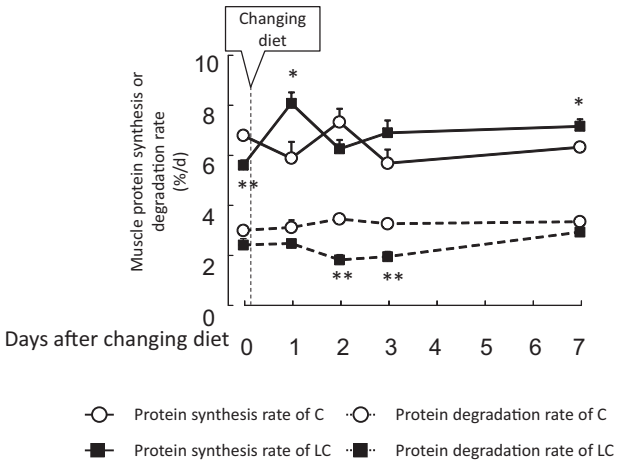

Fig. 3. Effect of changing dietary lysine levels from deficient to sufficient on the fractional protein synthesis rate and degradation rate of the skeletal muscle of rats. Values are means ± SE, n=6. Asterisks indicate significant differences versus control at a specific time point (P<0.01,* P < 0.05). Modified data from Ishida et al. (2011)**

after the change to the lysine-sufficient diet. Further, we reported that oral administration of lysine suppressed muscle proteolysis in the skeletal muscle of chicks (Nakashima et al. 2006). This suggests that a higher level of serum lysine might suppress proteolysis shortly after lysine sufficiency in the LC rats.

To determine the mechanism by which lysine sufficiency alters protein metabolism, we next measured the serum concentrations of IGF-I, IGF-binding protein (IGFBP)-3, and corticosterone, which play pivotal roles in protein metabolism regulation. IGF-I is a major regulator of the anabolic drive of nutrients and stimulates protein synthesis (Guler et al. 1989), while glucocorticoid stimulates proteolysis (Sacheck et al. 2004). When IGF-I binds to IGFBP-3, its priming binding protein, its half-life in the circulation is extended and its bioavailability is increased (Guler et al. 1989). Rats fed the low-lysine diet had lower serum concentrations of IGF-I and IGFBP-3 than the control rats until lysine sufficiency. Thereafter, the serum concentrations of IGF-I and IGFBP-3 of the LC rats rose rapidly: marked increases were observed, even on the first day after changing the diet (Table 1). This suggests that the dramatically increased IGF-I bioavailability in blood. In contrast to IGF-I, the concentration of serum corticosterone levels was elevated in rats fed the low-lysine diet (Table 1, $P=0.06$). This is in agreement with a previous report (Cree & Schalch 1985). However, when the diet was changed, the serum corticosterone levels decreased: serum corticosterone levels on day 17 (i.e. 3 days after changing the diet) was lower than that just before the dietary change. Moreover, on the third day after the diet change, the two groups no longer differed in terms of corticosterone level.

Table 1. Effect of changing dietary lysine levels from deficient to sufficient on serum IGF-I, IGFBP-3, corticosterone and lysine in rats. Values are means ± SEM, n=6. Within LC group, means without a common letter differ (P < 0.05). Asterisk indicates significant difference versus control at a time point (P<0.01,*P < 0.05). Modified data from Ishida et al. (2011)**

Experimental day	day 14		day 15		day 17	
Days after changing diet	0		1		3	
Treat	C	LC	C	LC	C	LC
IGF-I (ng/mL)	1173	498^{b**}	1142	788^{a}	1118	911^{a}
IGFBP-3 (ng/mL)	297	164^{b**}	307	250^{a}	274	298^{a}
Corticosterone (ng/mL)	749	1049^{a}	558	874^{ab*}	667	641^{b}
Lysine (mmol/L)	482	128^{c**}	433	572^{a**}	457	450^{b}

Thus, the compensatory growth caused by lysine sufficiency may be due to increased serum lysine level and changes in the levels of hormones that drive protein synthesis and degradation.

Compensatory growth of C2C12 myotubes induced by the combined effect of lysine sufficiency and modulation of IGF-I and glucocorticoid levels

IGF-I stimulates hypertrophy in myotubes and skeletal muscle (Adams & McCue 1998, Rommel et al. 2001, Sacheck et al. 2004), while glucocorticoid conversely induces proteolysis in myotubes and skeletal muscle (Hong & Forsberg 1995, Kayali et al. 1987, Sacheck et al. 2004). However, the roles played by IGF-I and glucocorticoid in compensatory growth remain unclear. Although our data suggested that lysine sufficiency-induced compensatory growth may be due, at least partially, to changes in circulating IGF-I and glucocorticoid levels that increase protein accumulation, it remains possible that the compensatory growth is induced by the recovery of circulating lysine levels and not by changes in the endocrine status.

However, it was not possible to distinguish between the contributions of the possible mechanisms in vivo in the rat or pig model because lysine sufficiency is always associated with changes in the IGF-I and glucocorticoid hormones. To separate the effects of lysine and hormones, an in vitro study using cultured cells was required. We determine whether myotubes exhibit compensatory growth using C2C12 myotubes in the presence of lysine sufficiency alone, i.e. increased concentration of lysine, and/or in combination with the modulation of IGF-I and glucocorticoid levels in the medium. To our knowledge, this is the first time compensatory growth phenomena have been studied in cultured muscle cells.

We prepared two media with different lysine levels, DMEM (Lys 0.8 mM) and a low-lysine medium (Lys 0.04 mM). The low-lysine medium contained the same components as DMEM except for lysine. The 0.04 mM lysine concentration in the low-lysine medium was based on our preliminary tests, which showed that reducing the lysine concentration of DMEM to 1/20 inhibited the growth of myotubes. IGF-I was added to the DMEM and low-lysine media at 50 ng/mL (denoted as IGF-) or 100 ng/mL (denoted as IGF+). The synthetic glucocorticoid, dexamethasone (Dex) was added to the two media at 1.0 μM (denoted as Dex-) and 1.5 μM (denoted as Dex-) The myotubes were fed for the first 18 h (donoted as the restriction period) with either DMEM/IGF-I+/Dex- or low-lysine/IGF-/Dex+. The concentrations of IGF-I and Dex in the low-lysine/IGF-/Dex+ medium were based on the serum hormone concentrations of pigs and rats. After the restriction period, the 18-h re-alimentation period started. The DMEM/IGF+/Dex- medium was replaced with the same fresh medium; this served as the positive control (PC). The low-lysine/IGF-/Dex+ medium was replaced with one of the following four media: fresh low-lysine/IGF-/Dex+ medium, which served as the negative control (NC), low-lysine/IGF+/Dex-, which served as IGF-I(+) and Dex(-), DMEM/IGF-/Dex+, which served as Lys(+), and DMEM/IGF+/Dex-, which served as Lys(+), IGF-I(+), and Dex(-). Cells incubated in the negative control medium for the first 18 h were treated with increased lysine concentration and/or increased IGF-I and decreased Dex concentrations for the next 18 h (Re-alimentation period).

Figure 4 shows the protein content in C2C12 myotubes. We determine the protein accumulation rate of cells during the last 18 h of culture to indicate compensatory protein accumulation. Although replacement of low-lysine/IGF-/Dex+ with DMEM/IGF-/Dex+ increased the protein content up to level equal to PC (Fig. 4), it did not affect the protein accumulation rate of the cells. In other words, increasing the lysine concentration alone was not sufficient to induce compensatory protein accumulation. Similarly, replacing the low-lysine/IGF-/Dex+ with the low-lysine/IGF+/Dex- medium also had no effect on the protein accumulation rate, which suggests that modulating the hormone concentrations in the medium alone was also not sufficient

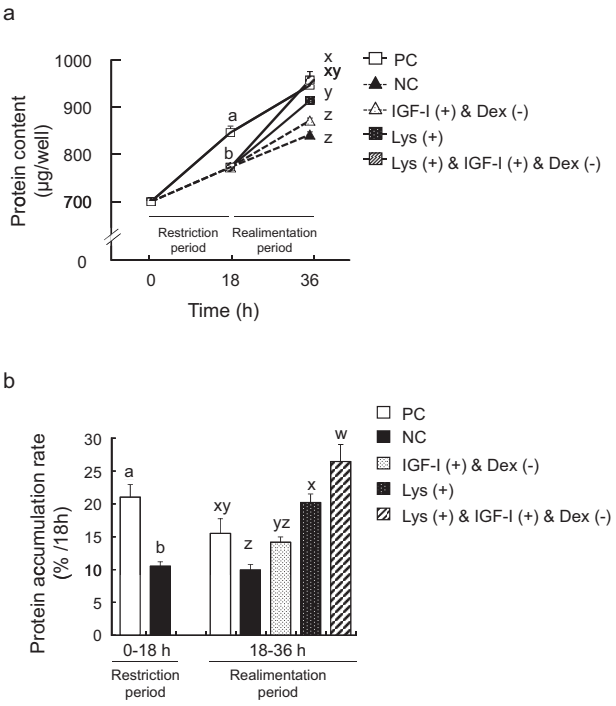

Fig. 4. Effects of increased lysine levels from deficiency to sufficiency and/or modulation of IGF-I and Dex in the medium on the protein content (A) and protein accumulation rate (B) of C2C12 myotubes. n = 6, Values are means ± SE. Different letters indicate significant differences among groups at a given time point (P < 0.05). Data from Ishida et al (2013)

to induce changes in protein content. However, when the low-lysine/IGF-/Dex+ medium was changed to DMEM/IGF+/Dex-, the cells showed a greater protein accumulation rate than those cultured continuously in the DMEM/IGF+/Dex- medium (Fig 4). Thus, C2C12 myotubes cultured in low-lysine, IGF-, and Dex+ conditions exhibited compensatory protein accumulation when they were subsequently cultured with the combination of increased lysine and IGF-I levels and decreased Dex levels. In other words, they exhibited in these combined conditions only. Yambayamba et al. (1996) and Therkildsen et al. (2004) report that when animals subjected to restricted feeding are fed ad libitum, they undergo compensatory growth and their plasma IGF-I concentrations change. Accordingly, in the present study, the combined modulation of lysine, IGF-I, and glucocorticoid levels is essential to induce compensatory growth in C2C12 myotubes and possibly also in animals after lysine sufficiency.

Conclusion

Our series of studies on compensatory growth after lysine deficiency revealed that both suppression of proteolysis and increased protein synthesis contributed to the compensatory growth that occurs after lysine sufficiency. Furthermore, simultaneous modulation of the serum lysine, IGF-I and glucocorticoid levels was needed to induce the change in protein metabolism associated with compensatory growth after lysine sufficiency. We infer that the induction of compensatory growth in animals after lysine sufficiency requires not only increased circulating lysine levels, but also changes in the endocrine status; the concentrations of IGF-I and glucocorticoids.

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