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# Apolipoprotein E knockout mice have accentuated malnutrition with mucosal disruption and blunted insulin-like growth factor I responses to refeeding☆

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**Abstract**

Apolipoprotein E (apoE) is synthesized mainly in the liver and in the brain and is critical for cholesterol metabolism and recovery from brain injury. However, although apoE mRNA increases at birth, during suckling, and after fasting in rat liver, little is known about its role in early postnatal development. Using an established postnatal malnutrition model and apoE knock-out (ko) mice, we examined the role of apoE in intestinal adaptation responses to early postnatal malnutrition. Wild-type and apoE-ko mice were separated from their lactating dams for defined periods each day (4 hours on day 1, 8 hours on day 2, and 12 hours thereafter). We found significant growth deficits, as measured by weight gain or tail length, in the apoE-ko mice submitted to a malnutrition challenge, as compared with malnourished wild type, especially during the second week of postnatal development ($P < .05$). In addition, apoE-ko animals failed to show growth catch-up after refeeding, compared with wild-type malnourished controls. Furthermore, we found shorter crypts and reduced villus height and area in the apoE-ko malnourished mice, compared with controls, after refeeding. Insulinlike growth factor 1 expression was also blunted in the ileum in apoE-ko mice after refeeding, compared with wild-type controls, which exhibited full insulinlike growth factor 1 expression along the intestinal crypts, villi, and in the muscular layer. Taken together, these findings suggest the importance of apoE in coping with a malnutrition challenge and during the intestinal adaptation after refeeding.


*Keywords:* ApoE; Malnutrition; IGF-1; Small intestine; Growing mice

## 1. Introduction

Apolipoprotein E (apoE), a 35-kd plasma protein synthesized mainly in the liver and in the brain, is critically involved in cholesterol transport and metabolism. Early studies identified apoE as a key component of plasma cholesterol homeostatic mechanisms [1,2]. ApoE binds with high affinity to

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lipoprotein particles in the plasma compartment and acts as a ligand for receptor-mediated endocytosis via multiple members of the low-density lipoprotein receptor family [1,3-5].

Several studies have highlighted the critical role of apoE in brain plasticity after injury (in vivo and in vitro models) [6-10]. On the other hand, to date, few studies have addressed the importance of apoE during early postnatal development, although apoE mRNA has been shown to increase at birth, during suckling, and after fasting in the rat liver [11]. In this study, we have investigated whether apoE null mice exhibit developmental deficits, focusing on intestinal healing, after a malnutrition challenge and refeeding, compared with wild-type controls. We have focused on the intestinal maturation during weaning, a transitional time when profound adaptations in small bowel morphology take place at the time of an introduction to a new diet regimen and when the small bowel structure is particularly prone to disruption [12,13].

Malnutrition is known to cause progressive changes in the intestinal permeability [14] and mucosa integrity, ultimately leading to hypoplasic villi and crypts and disruption of its biomechanical properties [15]. Most of these changes have been shown to be reversible after refeeding in a variety of refeeding protocols [16].

To investigate intestinal recovery from a malnutrition challenge, we have examined the intestinal catch-up after refeeding by monitoring morphological changes in villus height, area, and crypt length and insulin-like growth factor-I (IGF-1) expression in the ileal mucosa, which has been successfully used as a model of intestinal adaptation in rodents [17,18] and because distal parts of the small bowel are particularly vulnerable to a single period of food restriction [19].

Our findings suggest that apoE plays a critical role in intestinal healing, possibly by regulating IGF-1 actions in the ileum mucosa, raising the hypothesis that apoE might synergistically act with hormones and growth factors during the healing process in the intestinal mucosa. This animal model might provide further insights into understanding the relationship between apoE and nutritional interventions to improve the devastating effects of enteric infections and undernutrition in developing children in poor areas.

## 2. Methods and materials

### 2.1. Undernutrition model

ApoE knock-out (ko) mice were purchased from Jackson laboratories after being generated from B6.apoE$^{-/-}$ at the N10 backcross and constructed from B6.129 apoE$^{-/-}$ mice [20]. C57BL/6J wild types were purchased from Charles River Laboratories (Wilmington, MA). Either purchased pregnant mice or breeding pairs were used to obtain the study pups. Detectable pregnant mice (~12 days pregnant) were then caged individually, with free access to standard rodent chow and water, and were monitored daily for delivery (termed *day 0*). Newborn litters were adjusted to 7 to 8 pups. Undernutrition was induced by separating half the pups in each litter from their lactating dams for defined periods each day (4 hours on day 1, 8 hours on day 2, and 12 hours thereafter), according to protocol adapted from Calikoglu et al [21]. Both apoE-ko and wild-type mice underwent the same protocol for induction of undernutrition. This method has the advantage of providing littermate-control, well-nourished pups to compare with undernourished ones. At day 21, the study pups were removed from lactating dams and housed in a new cage, at which time they were fully weaned to a regular chow diet and water until the end point at day 27, ad libitum. Pups were euthanized after being refed with free access to chow diet (irradiated Harlan Teklad LM-485 for mice; Harlan Teklad, Madison, WI) for 7 days after weaning. Euthanasia was done by cervical dislocation after anesthesia with sodium pentobarbital (3-4 mg/100 g IP). Weight and tail length were recorded daily until euthanasia. A thermal pad was used to warm the pups during daily measurements (28°C ± 2°C). Protocols from this study were previously approved by the Institutional Animal Care and Use Committee at the University of Virginia.

### 2.2. Physical growth

Experimental mice were monitored carefully by daily inspection of weight gain and skeleton growth during the suckling time at days 1 to 20 (nourished and malnourished wild type [n = 11] and malnourished and nourished apoE-ko [n = 6]) and after refeeding (nourished and malnourished apoE-ko [n = 5] and nourished and malnourished wild type [n = 8]); at days 21 to 27, the skeletal growth was evaluated by assessing tail length by means of measuring gently the animal tail from the basis to the tip, using a digital caliber and a card board (to the nearest 0.1 mm). All measurements were conducted before starting the procedures of daily mice separation (8 to 10 AM). Care was taken to keep the same degree of handling during this process for both apoE-ko and wild-type pups.

### 2.3. Intestinal morphometry

Villus height and crypt depths were measured from slides stained with hematoxylin and eosin on a light microscope (BH-2, Olympus, Tokyo, Japan), n = 4 for each group, equipped with a high-resolution digital camera that was connected to a computer with an image capture program. Villus height was measured from the baseline to the villus tip. The crypt depth was measured from the baseline to the crypt bottom. The villous surface area was estimated by creating an apex-basis conical diagram on digital images at low magnification, and values were averaged and converted to a percentage of the phosphate-buffered saline control group, as described previously by Carneiro-Filho et al [22]. At least 10 clear longitudinal sections of villi and crypts were selected and counted for each sample (4 samples for each group). All morphometric measurements were done blindly with NIH Image J 1.34 S (National Institutes of Health, Bethesda, MD) analysis software.