

# How Is Vitamin A Absorbed by the Intestine upon Challenge with a Large Dose of Preformed Vitamin A?

Nuttaporn Wongsiriroj<sup>1,2</sup>, Roseann Piantedosi<sup>3</sup> and William S. Blaner<sup>2,3</sup>

<sup>1</sup>Institute of Molecular Biology and Genetics, Mahidol University, Bangkok, Thailand; <sup>2</sup>Institute of Human Nutrition and

<sup>3</sup>Department of Medicine, Columbia University, New York, USA.

## ABSTRACT

**Background:** Vitamin A deficiency (VAD) remains a public health problem causing morbidity and mortality in infants in developing countries. To correct VAD a large dose of preformed vitamin A (retinol) is administered. It is not clear how a large dose of retinol, one that may exceed the capacity of the intestine to absorb and esterify retinol is handled by the intestine. **Aims:** We are interested in understanding the mechanisms through which a large dose of retinol is processed by the intestine. To this end, we challenged several strains of mutant mice with a large dose of preformed vitamin A, resembling the dose administered to VAD infants. Specifically, we have studied mice lacking retinoid-binding protein (RBP) and enzymes thought to catalyze in the intestine retinol esterification (lecithin:retinol acyltransferase (LRAT) and diacylglycerol acyltransferase, type I (DGATI)) to gain understanding of vitamin A processing by intestine. **Results:** Our *in vitro* data demonstrate that DGATI esterifies retinol in an acyl-CoA dependent manner. Thus, DGATI acts as an acyl-CoA:retinol acyltransferase (ARAT), an enzyme which has been described in the literature but not previously identified. Studies of LRAT- and LRAT/DGATI-deficient mice, which have impaired capacity to esterify retinol, indicate that vitamin A is packaged primarily as free retinol into chylomicrons. Moreover, DGATI acts as an ARAT in the intestine. When *Lrat/Dgat1* double knockout mice were given a large dose of retinol some retinyl esters were still observed indicating other ARATs are also present. Studies of RBP/LRAT-deficient mice further indicate that free retinol is not absorbed bound to either RBP or discoidal high-density lipoprotein (HDL), as has been proposed in the older literature. **Conclusion:** Even in the face of challenge with a large dose of vitamin A that exceeds the capacity of the intestine to esterify vitamin A, the intestine is able to package the vitamin A as retinol in chylomicrons. Little free retinol gets taken up bound to RBP or discoidal HDL. Retinol is normally converted to retinyl ester by the actions of LRAT and DGATI, which acts physiologically as an ARAT. However, other ARATs are also present in the intestine and these become active when large doses of retinol are provided. In conclusion, our studies in animal models suggest that human infants given a large dose of vitamin A to prevent VAD will be able to absorb the vitamin A as retinol in chylomicrons even though the dose may exceed the capacity of the intestine to esterify the retinol. Furthermore, this may even render the dose more readily available to facilitate vitamin A-dependent actions in the body.

## INTRODUCTION

Details regarding the enzymes and other factors that are importantly needed to facilitate vitamin A absorption by the intestine are not well established (1). This lack of understanding holds both for normal dietary intake of vitamin A (when a physiological dose of vitamin A is ingested) and for when a large supplemental dose of vitamin A is administered to a patient (when a pharmacological dose of vitamin A is ingested). If we are to understand potential differences in how different groups absorb vitamin A, possibly arising through genetic polymorphisms present in human populations, it will first be necessary to understand what enzymes are essentially involved in mediating vitamin A uptake and metabolism within the small intestine.

Ultimately, all dietary vitamin A is packaged as retinyl ester along with other dietary lipids by the small intestine into chylomicrons (2). These chylomicrons are secreted from the intestinal cells into the lymphatic system and they eventually make their way into the general circulation. Once in the general circulation, triglyceride (fat) present in the chylomicrons is hydrolyzed to free fatty acids which are taken up by muscle and adipose tissue. The remainder of the chylomicron, referred to as the chylomicron remnant, is then taken up by the liver where the majority of the body's vitamin A reserves are stored.

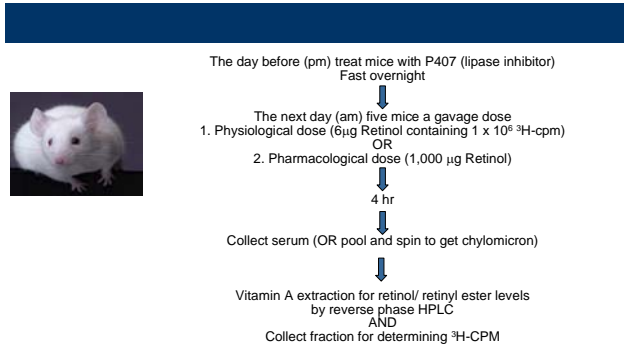
The literature suggests that the enzyme Lecithin:Retinol AcylTransferase (LRAT) is importantly involved in catalyzing retinyl ester synthesis in the small intestine and that Cellular Retinol-Binding Protein, type II (CRBP) facilitates retinyl ester formation from newly absorbed retinol (3). The literature also suggests that an as yet to be identified Acyl-CoA:Retinol AcylTransferase(s) (ARAT) also may play a role in this process, especially when very high doses of vitamin A are ingested (3). We and others recently demonstrated, through *in vitro* studies, Diacylglycerol AcylTransferase, type I (DGATI) as having ARAT activity (4-6).

Making use of unique mouse models that fail to express and hence lack LRAT (*Lrat*<sup>-/-</sup> mice), DGATI (*Dgat1*<sup>-/-</sup> mice) and both LRAT and DGATI (*Lrat*<sup>-/-</sup>/*Dgat1*<sup>-/-</sup> mice) we have explored the roles of LRAT, DGATI and other enzymes in facilitating intestinal vitamin A absorption in response to both physiologic (vitamin A levels encountered in a normal diet) and pharmacological (high vitamin A levels similar to those provided in supplements) oral challenges of vitamin A.

## OBJECTIVES

We are interested in understanding the intestinal uptake of dietary vitamin A and tissue sites of uptake of newly absorbed vitamin A. We have asked experimentally two questions. There are:

1. Does DGATI act as an ARAT *in vivo*, and if so is it the sole ARAT?
2. How physiological and pharmacological dose of retinol get absorbed in the intestine? What enzyme(s) does involve in these processes?



## RESULTS

### I. Physiological Dose of Retinol

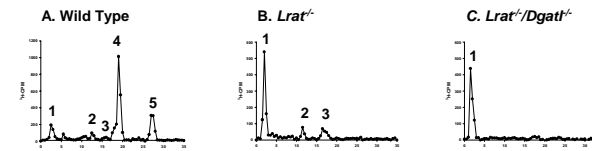


Figure 1. The distribution of [<sup>3</sup>H]retinoids profiles present in chylomicrons obtained from wild type (panel A), *Lrat*<sup>-/-</sup> (panel B) and *Lrat*<sup>-/-</sup>/*Dgat1*<sup>-/-</sup> (panel C) following administration of a physiological dose of retinol (6 µg retinol containing 1x10<sup>6</sup> <sup>3</sup>H-cpm) in peanut oil by gavage.

Panel A, B and C, the profiles demonstrate the distribution of <sup>3</sup>H-cpm. The vitamin A extractions were separated on a 5-µm 4.6 x 250-mm Ultrasphere C<sub>18</sub> column preceded by a C<sub>18</sub> guard column, using 70% acetonitrile, 15% methanol and 15% methylene chloride as the running solvent flowing at 1.8 ml/min. The numbers above the HPLC peaks indicate the following: 1, retinol; 2, retinyl linoleate; 3, retinyl oleate; 4, retinyl palmitate; and 5, retinyl stearate.

### II. Pharmacological Dose of Retinol

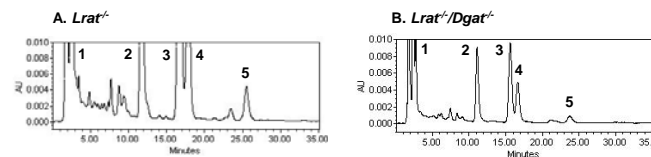


Figure 2. Comparison of reverse phase HPLC profiles showing the distribution of retinol and retinyl esters between serum obtained from *Lrat*<sup>-/-</sup> (panel A) and *Lrat*<sup>-/-</sup>/*Dgat1*<sup>-/-</sup> (panel B) following gavage administration of a pharmacological dose of retinol (1,000 µg retinol) in peanut oil.

Panel A and B, the profiles demonstrate the UV absorbance of vitamin A for pharmacological dose. For panel A and B, all profiles are scaled to reflect at full scale the same absorbance units (AU). The retinoid extractions were separated on a 5-µm 4.6 x 250-mm Ultrasphere C<sub>18</sub> column preceded by a C<sub>18</sub> guard column, using 70% acetonitrile, 15% methanol and 15% methylene chloride as the running solvent flowing at 1.8 ml/min. The numbers above the HPLC peaks indicate the following: 1, retinol; 2, retinyl linoleate; 3, retinyl oleate; 4, retinyl palmitate; and 5, retinyl stearate.

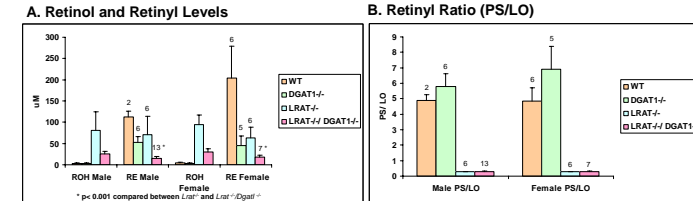
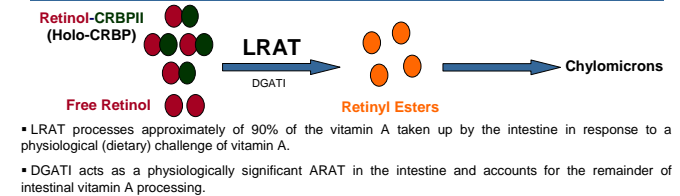


Figure 3. Retinol and retinyl ester levels (A) and ratios (B) in postprandial blood obtained from wild type, *Dgat1*<sup>-/-</sup>, *Lrat*<sup>-/-</sup> and *Lrat*<sup>-/-</sup>/*Dgat1*<sup>-/-</sup> following gavage administration of a pharmacological dose of retinol (1,000 µg retinol) in peanut oil.

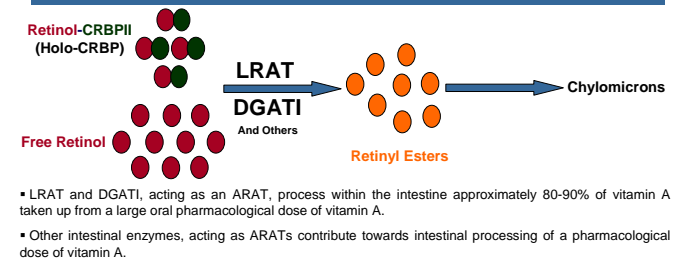
All values are given as means ± 1 S.D. The number of individual mice (n) used for each measurement is given above each total retinol values. The retinyl ester ratio values is expressed in the term of PS/LO (retinyl palmitate + retinyl stearate)/ (retinyl linoleate + retinyl oleate).

## CONCLUSIONS

### I. Physiological Dose of Retinol



### II. Pharmacological Dose of Retinol



### III. Summary

- LRAT, DGATI, as well as other enzymes contribute toward the enzymatic processing of vitamin A within the intestine.
- The importance of contribution of each of these enzymes varies markedly depending on the size of the vitamin A dose administered and could influence the efficacy of vitamin A intervention strategies.

## REFERENCES

1. N. Wongsiriroj, Wasantwisut E. and Blaner W.S. Vitamin A Deficiency in Children. *Clinical Studies in Medical Biochemistry* (Glew R. and Rosenthal M., eds) 3<sup>rd</sup> Ed. Oxford University Press.
2. W.S. Blaner, and Olson JA. Retinol and retinoic acid metabolism. *The Retinoids, Biology, Chemistry and Medicine* (Sporn MB., Roberts AB., and Goodman DS., eds) 2<sup>nd</sup> Ed. Raven Press, New York.
3. DE. Ong, Newcomer ME., and Chytil F. Cellular retinoid-binding proteins. *The Retinoids, Biology, Chemistry and Medicine* (Sporn MB., Roberts AB., and Goodman DS., eds) 2<sup>nd</sup> Ed. Raven Press, New York.
4. S. O'Byrne, Wongsiriroj N., Libien J., Vogel S., Goldberg I., Baehr W., Palczewski K. and Blaner W.S., Retinoid absorption and storage is impaired in mice lacking lecithin:retinol acyltransferase (LRAT). *J Biol Chem.* 2005. 280: 35647-57.
5. CL. Yen, Brown CH 4<sup>th</sup>, Monetti M., and Farese RV Jr. A human skin multifunctional O-acyltransferase that catalyzes the synthesis of acylglycerols, waxes, and retinyl esters. *J Lipid Res.* 2005. 46: 1502-11.
6. MD. Orland, Anwar K., Cronley D., Chu CH., Chen L., Billheimer JT., Hussain MM., and Cheng D. Acyl coenzyme A dependent retinoid esterification by acyl coenzyme A:diacylglycerol acyltransferase 1. *Biochim Biophys Acta.* 2005. 1737: 76-82.