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SBI Pharmaceuticals Co., Ltd.
Yamagata University, Faculty of Medicine

Presentation of Research Paper on 5-ALA by Yamagata University, Faculty of Medicine to *PLOS ONE*, a U.S. Scientific Journal
~ 5-ALA Deficiency Attenuates Mitochondrial Function and Impairs Glucose Tolerance ~

SBI Pharmaceuticals Co., Ltd., (Head office: Minato-ku, Tokyo; Representative Director & President: Yoshitaka Kitao), a subsidiary of SBI Holdings, Inc., engaged in the research and development of pharmaceuticals, health foods and cosmetics using 5-ALA (5-Aminolevulinic acid) (*1) and Yamagata University, Faculty of Medicine (Campus: Yamagata-shi, Yamagata; Faculty President: Hidetoshi Yamashita) carrying out joint research hereby announce that a research paper entitled "5-Aminolevulinic acid (5-ALA) deficiency causes impaired glucose tolerance and insulin resistance coincident with an attenuation of mitochondrial function in aged mice" presented by Professor Osamu Nakajima, Research Center for Molecular Genetics of Yamagata University, Faculty of Medicine, Institute for Promotion of Medical Science Research (Location: Yamagata-shi, Yamagata; Director of Management Committee: Takamasa Kayama), has been published in *PLOS ONE*,, a U.S. scientific journal as follows:

Name of Journal	PLOS ONE
Title	5-Aminolevulinic acid (5-ALA) deficiency causes impaired glucose tolerance and insulin resistance coincident with an attenuation of mitochondrial function in aged mice
URL	http://journals.plos.org/plosone/article?id=10.1371/journal.pone.0189593
Abstract	In vertebrates, the initial step in heme biosynthesis is the production of 5-aminolevulinic acid (5-ALA) by ALA synthase (ALAS). ALA formation is believed to be the rate-limiting step for cellular heme production. Recently, several cohort studies have demonstrated the potential of 5-ALA as a treatment for individuals with prediabetes and type-2 diabetes mellitus. These studies imply that a mechanism exists by which 5-ALA or heme can control glucose metabolism. The *ALAS1* gene encodes a ubiquitously expressed isozyme. Mice heterozygous null for *ALAS1* ($A1^{+/-}$s) experience impaired glucose tolerance (IGT) and insulin resistance (IR) beyond 20-weeks of age (aged $A1^{+/-}$s). IGT and IR were remedied in aged $A1^{+/-}$s by the oral administration of 5-ALA for 1 week. However, the positive effect of 5-ALA proved to be reversible and was lost upon termination of 5-ALA administration. In the skeletal muscle of aged $A1^{+/-}$s an attenuation of mitochondrial function is observed, coinciding with IGT and IR. Oral administration of 5-ALA for 1-week brought about only a partial improvement in mitochondrial activity however, a 6-week period of 5-ALA treatment was sufficient to remedy mitochondrial function. Studies on differentiated C2C12 myocytes indicate that the impairment of glucose metabolism is a cell autonomous effect and that 5-ALA deficiency ultimately leads to heme depletion. This sequela is evidenced by a reduction of glucose uptake in C2C12 cells following the knockdown of *ALAS1* or the inhibition of heme biosynthesis by succinylacetone. Our data provide *in vivo* proof that 5-ALA deficiency attenuates mitochondrial

	function, and causes IGT and IR in an age-dependent manner. The data reveals an unexpected metabolic link between heme and glucose that is relevant to the pathogenesis of IGT/IR.

This research elucidated that the deficiency in the production of 5-ALA in aged mice attenuated mitochondrial function and was one of the causes of IGT and IR. The continuous administration of 5-ALA to those mice demonstrated efficacy against IGT and IR. It is expected that the mechanism of cause of IGT and IR by 5-ALA deficiency will be investigated in human, and this will lead to the clarification of the mechanism in the effect of 5-ALA against type-2 diabetes and so on.

(*1) 5-aminolevulinic acid (5-ALA): An amino acid produced in mitochondria. It is an important substance that serves as a functional molecule related to energy production in the form of heme and cytochromes, and its productivity is known to decrease with age. 5-ALA is contained in food such as shochu lees, red wine and Asian ginseng. It is also known as a material forming chloroplasts in plants.

(*2) C2C12 cell: Myoblasts derived from mouse striated muscle, often used in the in-vitro experiments of skeletal muscle differentiation. Recently there are a variety of papers reporting the degeneration of skeletal muscle caused by glucose load, such as abnormal increase of type IIB fiber. In-vitro experiments have been conducted by using C2C12 myoblasts, focusing on growth and differentiation, and have explored the effects of glucose and insulin under culture conditions.

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For further information, please contact:
SBI Pharmaceuticals Co., Ltd.: Corporate Planning Dept.,
Tel: 03-6229-0095 E-Mail: info_ala@sbigroup.co.jp
Yamagata University, Faculty of Medicine, Institute for Promotion of Medical Science Research,
Research Center for Molecular Genetics, Professor Osamu Nakajima
Tel: 023-628-5901 E-Mail: nakajima@med.id.yamagata-u.ac.jp