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RESEARCH ARTICLE

TDO EXPRESSION INCREASES DURING STARVATION IN MOUSE LIVER

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ABSTRACT

The essential amino acid L-tryptophan (Trp) is the important precursor of a number of biologically active metabolites. Tryptophan 2,3-dioxigenase (TDO) is a liver-specific enzyme for tryptophan catabolism. Although the regulation of TDO expression is understood to involve glucagon *In vitro*, it is largely unknown *in vivo*. To investigate the regulation of TDO expression in mouse liver during starvation, I performed real-time RT-PCR and Western blot analysis. At first, I assessed the changes of body weights and the liver histology with Periodic acid-Schiff (PAS) staining during starvation, and found that starvation lead into depletion of glycogens and glycoproteins and so on. I also observed that *Tdo* mRNA and TDO protein were increasedduring starvation by comparing the normal controls. I thus conclude that starvation increases the hepatic TDO expression. Tryptophan and/or its catabolism(s) could play important roles in liver and whole body during starvation.

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INTRODUCTION

L-tryptophan (Trp) is one of the essential amino acid and the important precursor of a number of biologically active metabolites, such as vitamin B, $NAD^+(P)^+H$, the neuroactive kynurenine, quinolinic acid, the 5-hydroxytryptamine (5-HT or serotonin), and the pineal hormone melatonin. Almost only 1% of dietary Trp is utilized for protein synthesis, because the amount of protein degraded is matched exactly by that synthesized in a person in nitrogen equilibrium, with the bulk of Trp being available for metabolism (Bender, 1983). Tryptophan 2,3-dioxigenase (TDO) is a liver-specific enzyme for tryptophan catabolism. The importance of TDO in Trp degradation is best shown by the finding that deletion of the mouse TDO gene elevates plasma Trp (Kanai *et al*., 2009). Most studies of the hepatic kynurenine pathway have focused on changes in the first and rate-limiting enzyme TDO and some subsequent enzymes in the pathway induced by Trp, various drugs and other chemicals, and nutritional deficiencies (Badawyand Bano, 2016). The enzyme appears 2 weeks after birth and reaches the adult level after 4-5 weeks in the rat liver (Nakamura *et al*., 1987). TDO is found only in mature liver and induced to express by glucocorticoids (Nakamura *et al*., 1987; Danesch *et al*., 1987). It is also well established that pancreatic hormones such as glucagon induce the production of TDO, whereas insulin suppresses this production in rat primary hepatocytes (Nakamura *et al*., 1980). Although it is understood to involve glucocorticoid *in vitro*, the TDO expression is largely unknown *in vivo*. Here, I report the upregulation of TDO expression in mouse liver during starvation.

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MATERIALS AND METHODS

Experimental animals and Liver histology

10-week-old male mice were housed in a room with controlled light/dark cycle, humidity, and temperature, and allowed *ad libitum* access to food and water. The acquisition, care, housing, use, and disposition of the animals were in compliance with the institutional laws and regulations of the Osaka University Graduate School of Medicine. Liver removed at each autopsy was fixed in cold 10%-buffered formalin for 24 h respectively. Transversally trimmed liver tissues were submitted to a routine process for paraffin embedding (Kato *et al*., 2010; Kato *et al*., 2014). The renal sections were prepared, deparaffined, stained with PAS (Periodic Acid–Schiff).

RNA purification and quantitative real-time RT-PCR

Total RNA was purified from the livers of mice using a TRIzol reagent (Invitrogen) according to the manufacturer's instructions. Quantitative real-time RT-PCR was carried out and mRNA levels were calculated as described previously (Kanai *et al*., 2009; Danesch *et al*., 1987).

Western blotting

Western blot analyses of liver lysates were performed using anti-TDO antiserum (Niimi *et al*., 1983).

RESULTS

Before testing TDO expression level, I measured the changes of body weights (BWs) during starvation (Figure 1A). At 24 h starvation, the BWs of mice decreased into 91% compared with normal states. At 48 h, the BWs decreased further 85%. Moreover, I assessed the histological analysis in liver with Periodic acid-Schiff (PAS) staining (Figure 1B).

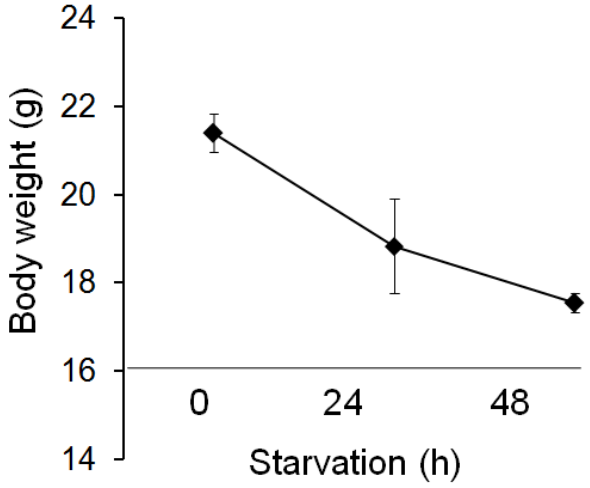


Figure 1A.

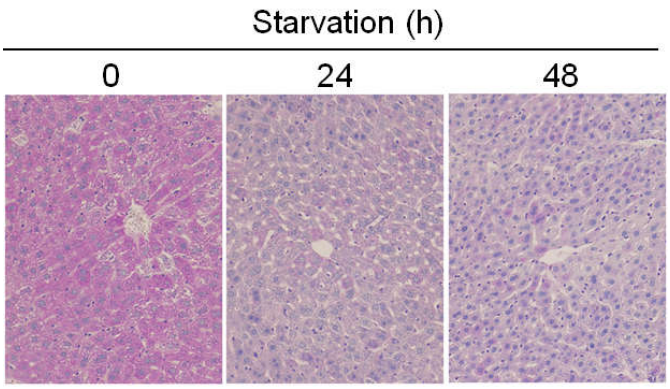


Figure 1B.

PAS is a staining method used to detect polysaccharides such as glycogen, and mucosubstances such as glycoproteins and glycolipids in tissues. Under normal state, liver section was sufficient for PAS positive. However, after 24 h starvation, PAS stain became notably weak. These findings show the starvation leads into depletion of glycogens and glycoproteins and so on. I investigated the hepatic TDO changes during starvation. At first, I studied the *Tdo* mRNA levels by using real-time RT-PCR. Comparing the normal controls, *Tdo* mRNA was increased at both 24 and 48h (Figure 2A). Furthermore, I analyzed the TDO protein expression at 24 and 48 h by Western blot, and found the increase of TDO protein levels by starvation (Figure 2B). I measured the band intensity of TDO normalized by β-actin, and confirmed the significant difference (Figure 2B). Therefore, I conclude that starvation increases the hepatic TDO expression.

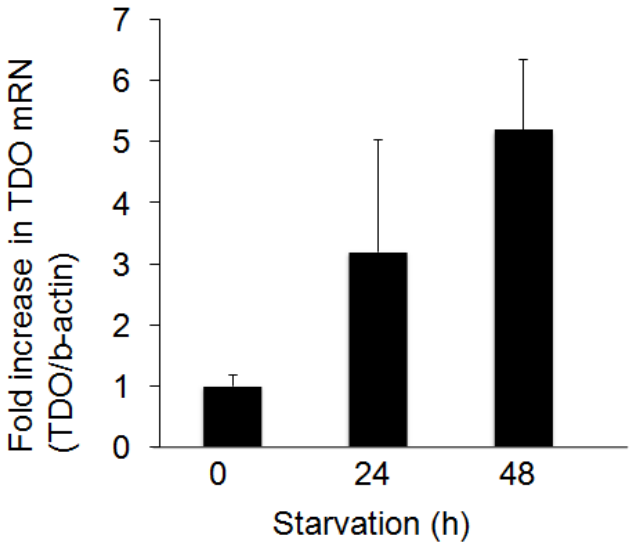
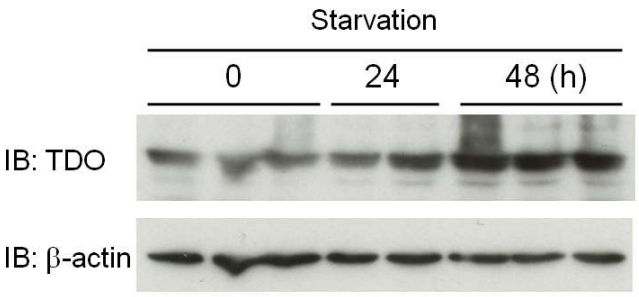


Figure 2A.



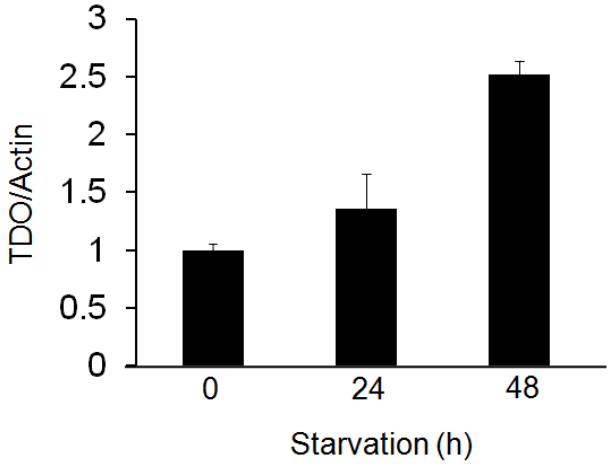


Figure 2B.

DISCUSSION

In recent year, the regulatory mechanisms for TDO expression become a focus field on many scientists. The hepatic TDO level was upregulated by starvation in the present study. The degradation of tryptophan is also catalyzed by the indoleamine 2,3-dioxygenase (IDO) (Prendergast *et al*., 2014). Both TDO and IDO catalyze the first and rate-limiting step of tryptophan oxidation yielding kynurenine, and a high degree of structural similarity in the area around the catalytic site, although they have no homology sequence (Zhang *et al*., 2007; Batabyal and Yeh, 2007). TDO and/or IDO contribute to tumor-associated immune suppression. Several tumors express TDO, such as bladder cancer, hepatocellular carcinoma, melanoma, a

glioblastoma (Opitz *et al*., 2011; Pilotte *et al*., 2012). TDO expression by tumors could prevent their rejection (Pilotte *et al*., 2012). On the other hand, IDO is upregulated by inflammatory cytokines such as type I and II interferons, and might control immune responses (Munn and Mellor, 2007). Of note, depletion of tryptophan induces signaling events in T cells, leading to anergy and apoptosis; however, active immunomodulation by accumulating tryptophan catabolites, most notably kynurenine, appears to play an equally important role (Platten *et al*., 2012). Therefore, kynurenine might be a key catabolite for dealing on starvation. One of amino acid sensors is the mechanistic target of rapamycin complex 1 (mTORC1) (Laplante and Sabatini, 2012; Avruch *et al*., 2009). Activated mTORC1 regulates the protein synthesis (for cell proliferation, survival, and mobilization), lipogenesis, and autophagy (Laplante and Sabatini, 2012; Efeyan et al., 2013). Although the precise molecular mechanism by which mTORC1 senses intracellular amino acids has not yet been fully elicdated, amino acid-dependent mTORC1 activation is known to require Rag GTPases (Efeyan et al., 2013;Sancak *et al*., 2008; Kim *et al*., 2008) and vascular H^+-ATPase (Zoncu *et al*., 2011) on the lysosomal surface. Amino acids, particularly Leu and Arg, have been shown to activate mTORC1 (Hara *et al*., 1998), but it remains unknown whether mTORC1 activation is involved in sensing other amino acids including tryptophan. TDO induction might decrease intracellular tryptophan. Therefore, decreased tryptophan could suppress the mTORC1 activity, and inhibit the cell proliferation. Additionally, upon nutrient starvation, autophagy is induced to promote cell survival (Takeshige *et al*., 1992; Tsukada and Ohsumi, 1993). Therefore, tryptophan deprivation could lead into autophagy activation, and bring about cell survival.

In summary, the mouse TDO increase during starvation in liver. TDO is an enzyme for tryptophan catabolism, and an increase of TDO expression means tryptophan depletion. Tryptophan depletion and/or its catabolism(s) could play important roles in liver and whole body during starvation.

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