**Asprosin, a fasting-induced hormone, can be synthesized and purified in bacterial and mammalian systems**

Pallavi Sharma,1 Richard Breyer, PhD2

Northeast Ohio Medical University1

Department of Medicine, Division of Nephrology and Hypertension, Vanderbilt University2

(440)-334-7934, psharma1@neomed.edu, MS-4

The release of hepatic glucose serves an important role in the management of type II diabetes mellitus and normal systemic glucose levels. Various hormones are known to regulate circulating glucose homeostasis. Recently, a fasting-induced protein hormone termed asprosin has been isolated as a cleavage product of profibrillin. It is released from white adipose tissue and increases hepatic glucose release, thereby increasing systemic glucose levels. The method of action of asprosin is, however, incompletely characterized. To test the hypothesis that asprosin can be synthesized, purified, and eventually tested for biologic activity in liver cells, we first cloned recombinant asprosin in bacterial and mammalian systems. 6xHis tagged asprosin DNA was initially synthesized into a pUC57 vector and then subcloned into pcDNA3.1 for mammalian expression and pET T7 for bacterial expression. After expression, the protein was purified by using a Co2+ immobilized metal affinity chromatography (IMAC) column and eluted with imidazole, a His analog. The purity of the protein was analyzed by SDS-PAGE on 4-20% polyacrylamide gels and stained sequentially with Coomassie and silver stains. The purification showed an isolated band of approximately 18kDa, indicating that asprosin protein was synthesized and purified. The BCA analysis of asprosin indicated protein was synthesized homogenously with a concentration of 120μg/mL and a total yield of 0.75mg from a 250mL bacterial culture. Based on the results, we have expressed and purified asprosin in both bacterial and mammalian systems. Future studies aim to confirm the activity of asprosin protein in primary hepatocytes and/or hepatocyte cell lines (eg. HepG2 cells), as well as test systemic glucose fluctuations in vivo. Purification of asprosin after expression provides a source of recombinant asprosin to test the pathophysiologic effects of this newly described hormone, allowing a better understanding of the hormone and its association with type II diabetes.