# 学 位 論 文 の 要 旨

氏名　　田中　小百合

学 位 論 文 名　Effects of Uremic Toxin *p*-Cresol on Proliferation, Apoptosis, Differentiation and Glucose Uptake in 3T3-L1 Cells

発 表 雑 誌 名　Artificial Organs
（巻，初頁～終頁，年）　38(7):566-571,2014

著 者 名　Sayuri Tanaka, Shozo Yano, Abdullah M. Sheikh, Atsushi Nagai, Toshitsugu Sugimoto

## 論 文 内 容 の 要 旨

## INTRODUCTION

Malnutrition and sarcopenia commonly seen in chronic dialysis patients may resulted from restriction of protein intake and hypercatabolism. Among this population, body fat ratio was reported to be increased as compared with muscle mass. However, previous prospective study revealed that body fat mass was markedly decreased 2 years after the initiation of dialysis therapy. Moreover, dialysis patients with obesity have a better survival rate and a less cardiovascular (CV) death rate, compared with lean patients, which is so-called "reverse epidemiology". It is also known that chronic kidney disease (CKD) patients, even if they have neither obesity nor diabetes, exhibit the insulin resistance, which is likely to have a close relationship with arteriosclerosis and CV event. According to previous studies regarding the insulin resistance in renal failure patients, glucose uptake and glyconeogenesis in the liver are similar to those in healthy subjects whereas glucose uptake in the muscle and adipose tissue falls to 60% of the counterpart. Although uremic toxins can be involved in the insulin resistance and CV disease risk in this population, little is known about their pathogenesis so far. While *p*-cresol, one of uremic toxins is hardly detected in blood from healthy subjects, the blood concentration in

dialysis patients is reported to be 24mg/L (≒200μM). The blood *p*-cresol level is hardly reduced even after hemodialysis session because of highly protein-binding property. Although it has been reported that its concentration is highly associated with the risk of CV event, the precise mechanisms remain unknown. Thus, we focused on this uremic toxin to examine the effects in adipocytes and the precursors.

## MATERIALS AND METHODS

We cultured mouse preadipocyte cell line, 3T3-L1 cells, which were differentiated into mature adipocytes with 500μM IBMX, 250nM dexamethasone, 10μg/ml Insulin after 90% confluency. Cell proliferation was determined by cell count and Brd-U antibody detection method after plating. The maturity of adipocyte was determined by oil red-O staining. PPARγ mRNA expression was quantified by real-time PCR. Apoptosis of the cells were analyzed using a ELISA-based method. Glucose uptake was examined in the presence and absence of insulin using radiolabeled 2-deoxyglucose. Effects of *p*-cresol were evaluated in various concentrations (2-200μM).

## RESULTS AND DISCUSSION

In the cell count experiments, the number of 3T3-L1 cells treated with 100-200μM *p*-cresol was significantly decreased at day 3 and day 7. Brd-U antibody detection showed similar results, suggesting that *p*-cresol disturbed normal cell cycle. Oil red-O staining at day 7 showed that the number of mature adipocytes was decreased by the treatment with 200μM *p*-cresol. As consistent with this finding, apoptotic cell number at day 7 was increased by the treatment with 100-200μM *p*-cresol. Two hundred μM *p*-cresol decreased mRNA expression of PPARγ even when corrected with GAPDH mRNA level. $^{3}$H-labeled 2-deoxyglucose uptake was remarkably reduced by 200μM *p*-cresol in the presence and absence of insulin, mainly because of decreased number of mature adipocytes.

We investigated whether or not uremic toxins lead to the insulin resistance. In the present

study, we found that *p*-cresol of the blood level reported in dialysis patients inhibited proliferation, maturation and glucose uptake of 3T3-L1 cells, suggesting that *p*-cresol may be responsible for the loss of fat mass and the development of insulin resistance in spite of the absence of obesity in chronic dialysis patients. Moreover, the toxic effect was also accompanied with an increase in apoptosis and decrease in the number of mature adipocytes, which are most likely attributable to the reduced glucose uptake. In addition, high concentration of *p*-cresol inhibited an insulin-induced glucose uptake. This suggests that *p*-cresol reduced glucose uptake due to not only decreased number of mature adipocytes but also attenuated insulin sensitivity.

In our study, we firstly found that *p*-cresol might be involved in reduced fat mass and the development of insulin resistance. Hence, these findings are compatible to the "reverse epidemiology", where lean patients have higher mortality rate than obese patients undergoing dialysis therapy.

## CONCLUSION

*p*-cresol inhibited proliferation and differentiation, and induced apoptosis in 3T3-L1 cells. These findings indicate that the accumulation of uremic toxins may induce the reduction of adipose tissue, insulin resistance, and eventually poor prognosis in chronic dialysis patients. New therapeutic methodologies, which target reduction or removal of uremic toxins such as *p*-cresol, may lead to better prognosis in dialysis patients.

論文審査及び最終試験又は学力の確認の結果の要旨

| ㊙ ・ 乙 | 氏 名 | 田中　小百合 |
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| 学位論文審査委員 | 主 査 | 土屋　美加子　印 |
| | 副 査 | 熊倉　俊一　印 |
| | 副 査 | 小林　裕太　印 |

論文審査の結果の要旨

慢性透析患者では体重や体脂肪の減少が起こる一方でインスリン抵抗性が高いことが知られている。申請者らは、尿毒素の一つである *p*-cresol が脂肪細胞に与える影響を明らかにする目的で、マウス脂肪前駆細胞 3T3-L1 を用いて、*p*-cresol の増殖への効果に加え分化過程における 1)アポトーシス、 2)脂肪細胞への分化マーカーである PPARγmRNA の発現、3)脂肪滴を有する細胞への成熟および 4)グルコース取り込みへの効果を検討した。その結果 *p*-cresol は前駆細胞の増殖を抑制し、脂肪細胞への分化過程に存在することでアポトーシスを増加させる一方、PPARγmRNA の発現、脂肪滴を有する細胞およびインスリン依存性グルコース取り込みを減少させた。これらの結果は慢性透析患者の血中に高濃度に存在する尿毒素 *p*-cresol が、脂肪細胞に影響を及ぼすことでインスリン抵抗性を惹起する可能性を示唆するものであり、慢性透析における体脂肪減少ならびにインスリン抵抗性出現のメカニズム解明に資する可能性がある。

最終試験又は学力の確認の結果の要旨

申請者は、尿毒素の一つ *p*-cresol が 3T3-L1 細胞の増殖、分化に及ぼす影響を検討し、この物質が慢性透析患者における体脂肪減少、インスリン抵抗性に関与する可能性を示した。関連分野の知識も有しており、学位授与に値すると認める。　（主査：土屋美加子）

申請者は、尿毒症患者の血中で上昇する *p*-cresol がマウス脂肪前駆細胞の細胞死を誘導するとともに、細胞の分化・成熟と細胞内へのグルコース取り込みを抑制することを明らかにし、尿毒症患者の脂肪組織減少とインスリン抵抗性獲得の機序を示唆した。関連分野の豊富な学識も有しており、学位授与に値すると判断した。　（副査：熊倉　俊一）

申請者は、透析患者に多い心血管死に関連する可能性のある尿毒素の一つ *p*-cresol が培養脂肪細胞への抑制効果を示すことを明らかにした。透析患者の合併症の抑制につながる可能性のある研究成果と考えられる。公開審査では的確に質疑応答し、関連知識も豊富であることから学位授与に値すると判断した。　（副査：小林　裕太）

（備考）要旨は、それぞれ４００字程度とする。