<제3협동과제 : 대사성 질병 기전 및 Ketosis 질병 간이 진단키트 개발>

1. 이론 및 실험 방법

가. 케토시스 저항성 증진 후보물질 개발: Ketosis Cell Model을 이용한 분자생물학적, 생화학적 기전 규명

① 구축된 Ketosis Cell Model을 이용하여 FL83B cells에서의 TA, Momilactone B, SDG (Secoisolariciresinol diglucoside)의 세포독성 분석하였다.

② Ketone body (β-hydroxybutyrate) (BHB) estimation

- 4 groups were categorized as low glucose treated control group (ketosis condition), high glucose treated group (normal condition), and two ketosis group (low glucose) treated with TA, Momilactone B, SDG (Secoisolariciresinol diglucoside).

③ Western blot analysis

- The whole cell lysate prepared from 4 groups of cells such as low glucose treated control group, high glucose treated normal group, and two ketosis group (low glucose) treated with TA, Momilactone B, SDG (Secoisolariciresinol diglucoside).

④ RT-PCR analysis

- The mRNA isolated from 4 groups of cells such as low glucose treated control group, high glucose treated normal group, and two ketosis group (low glucose) treated with TA, Momilactone B, SDG(Secoisolariciresinol diglucoside). Analysis of Angiopoetin like protein-3 (ANGPTL3) was conducted as per Hong-Bo Xiao et al., The expression level of ANGPTL3, LPL should upregulate in ketosis condition. Obtained result showed the downregulated expression in high glucose group when comparing with the low glucose ketosis group.

⑤ Electrophoretic Mobility Shift Assay

- The whole cell lysate and nuclear extracts were prepared from 4 groups of cells such as low glucose treated control group, high glucose treated normal group, and two ketosis group (low glucose) treated with TA, Momilactone B, SDG(Secoisolariciresinol diglucoside). Expression levels of STAT5b, pSTAT5 and β-actin were analyzed in whole cell lysate. And expression levels of STAT5b and pSTAT5 were also analyzed.

나. 케토시스 간이 검사시약 개발: 우유에서의 조기 진단기술

① 검사 원리

- Ketone bodies는 Acetone, Acetoacetic acid, BHBA(β-hydroxybutyric acid)으로 구성되는