Assessment of NF-κB-SN50 Effect on Adipose TNF-α and AGT Secretion and Expression as A therapeutic Agent

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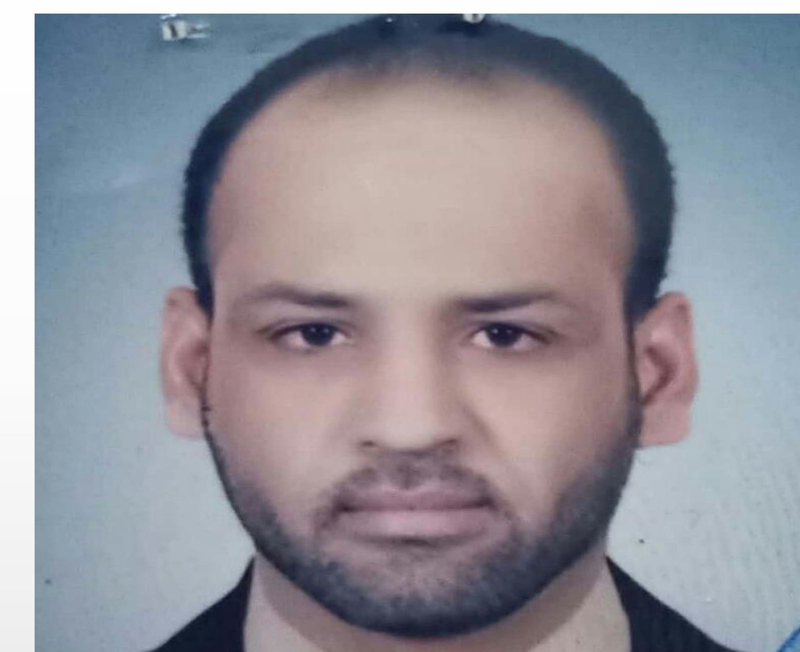


Lotfi Bin Dahman

Introduction

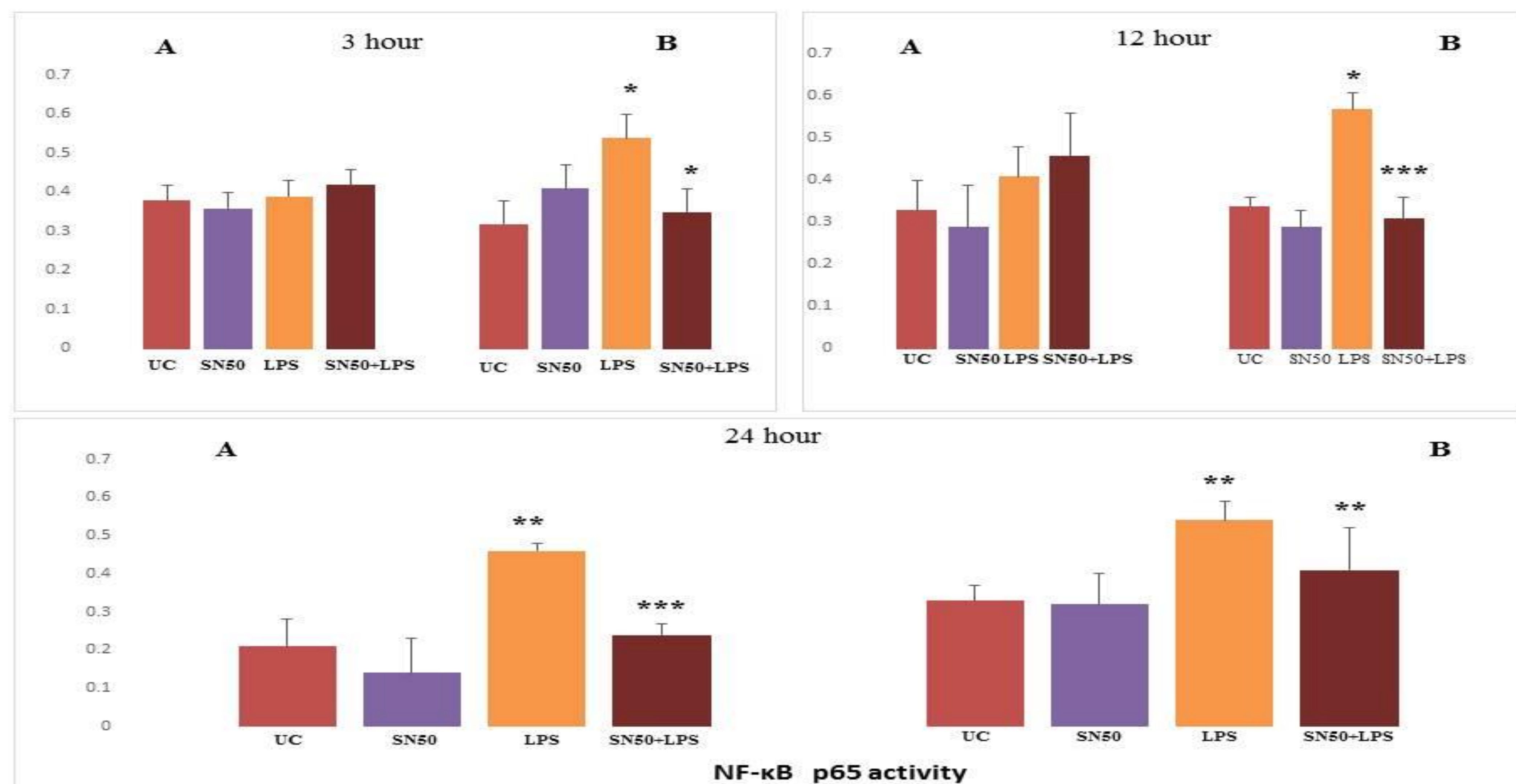
Pro-inflammatory cytokines and adipokines upregulation through NF-κB activation in adipose tissue has been considered to have an important function in the pathogenesis of obesity-related hypertension. This study was aimed to ascertain the effect of NF-κB inhibitor, (SN50) on TNF-alpha and AGT secretion and expression in mediating the anti-inflammatory effect through its effects on NF-κB activity in human adipose tissue.

Methods

Primary human adipocytes were isolated from 20 subjects among 10 overweight and 10 obese with and without hypertension, cultured, then treated with 10 ng/ml LPS, with and without NF-κB inhibitor, SN50 (50 μg/ml) at different time points. TNF-α secretion and NF-κB p65 activity were detected in supernatants extracted from cultured cell treated and untreated with LPS and SN50 by ELISA. NF-κB p65, TNF-α and AGT proteins expression were detected by western blot. TNF-α and AGT gene expression was detected in cells and performed using quantitative real time-PCR. The study was carried out at the Obesity Research Center, King Saud University, Riyadh, KSA.

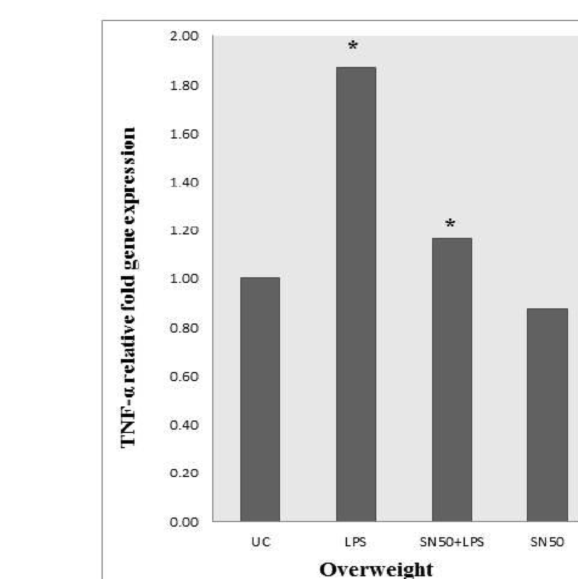
Results

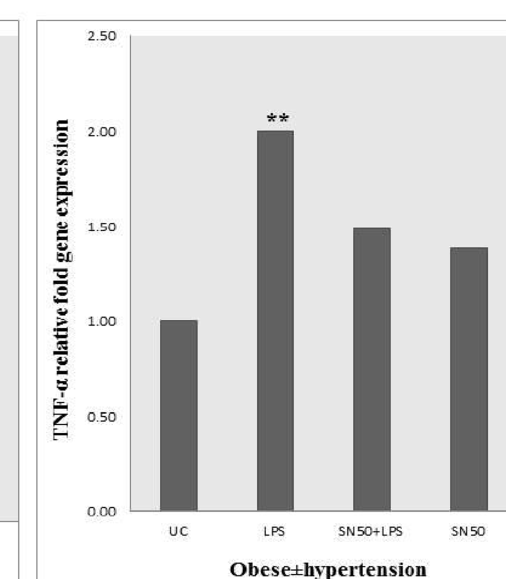
Treatment of AbdSc adipocytes with LPS caused a significant increase in NF-κB p65 in overweight and obese, while, SN50-NF-κB inhibitor causes a reduction of NF-κB p65 in overweight and obese persons at all time points. Treatment of AbdSc adipocytes with LPS caused a significant increase in TNF-α secretion in overweight and obese subjects at all time points, whereas, SN50 leads to a decrease in TNF-α secretion at 3 and 12 hours. Treatment of AbdSc adipocytes with LPS caused increased TNF-α and AGT gene expression twofolds compared with untreated cells, whereas, in the presence of SN50, it reduces mRNA AGT levels in both groups.



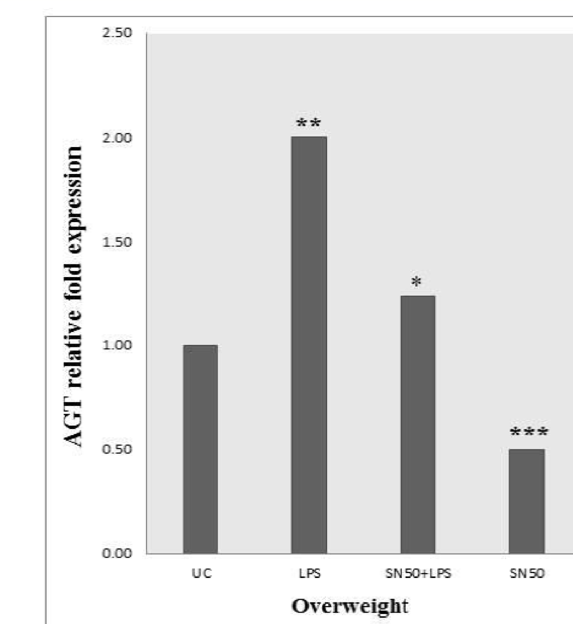
Conclusion

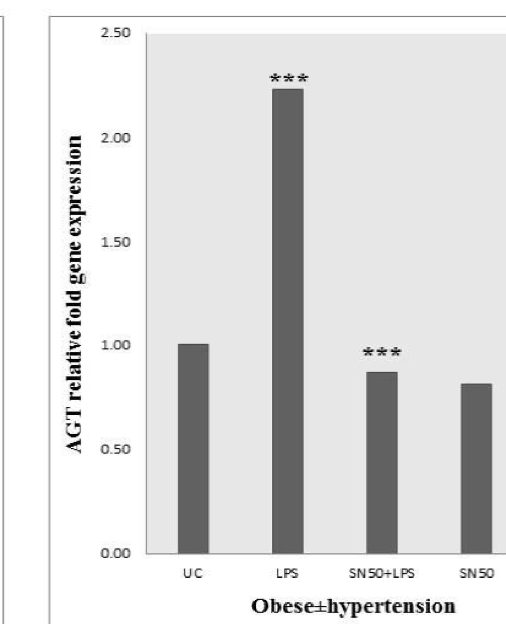
Taken together these adipokines with NF-κB activation may represent important biomarkers to evaluate hypertension risk, as well as provide a mechanistic insight into the pathogenesis of obesity-related hypertension



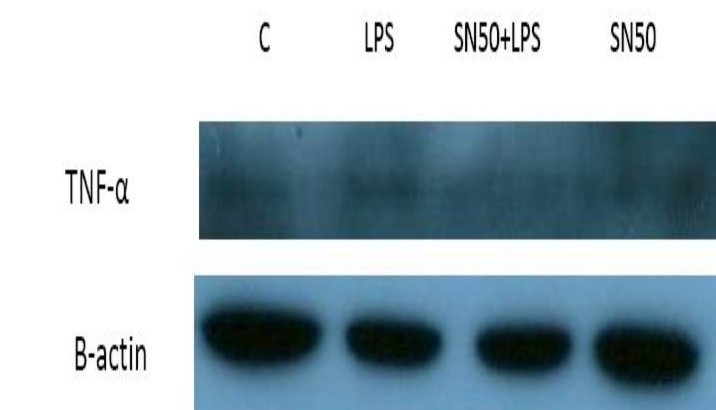


Total RNA was isolated from AbdSc adipocytes from overweight (n=10) and obese with and without hypertension (n=10), treated with LPS (10 ng/ml) in the absence and presence of SN50 (50 μg/ml) at 12 hours. Quantitative RT-PCR was performed using premade TaqMan probe for TNF-α. The quantitative fold changes in mRNA expression were determined as relative to 18S mRNA levels in each corresponding group and calculated using $2\text{-}\Delta\Delta^{CT}$ method. Statistical analysis was undertaken using an independent sample t-test. $p<0.05$ was considered as a significant versus untreated cells. * = p value 0.02, ** = p value 0.002 (Overweight); ** = p value 0.005 (Obese±hypertension).

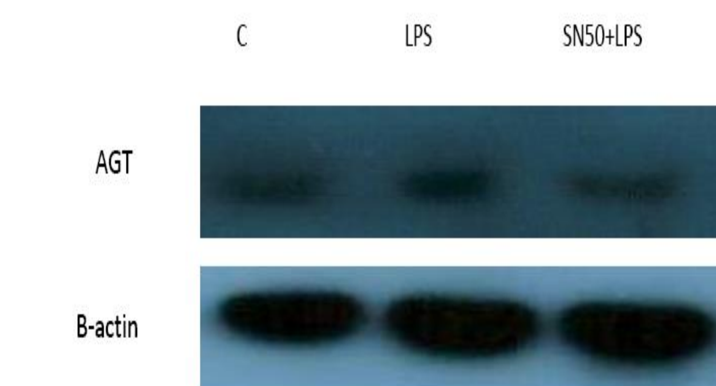




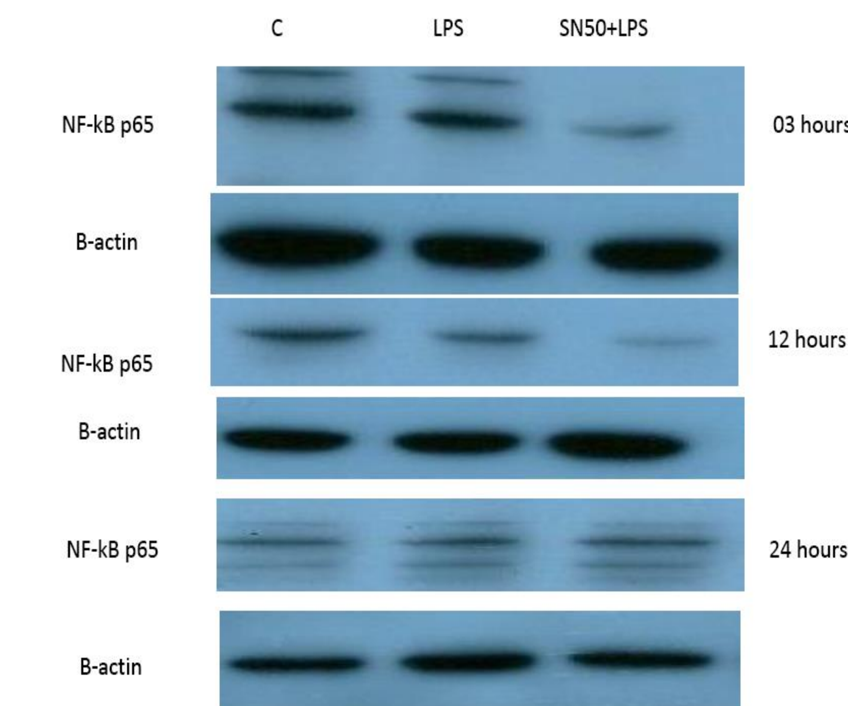
Total RNA was isolated from AbdSc adipocytes from overweight (n=10) and obese with and without hypertension (n=10) treated with LPS (10 ng/ml) in the absence and presence of SN50 (50 μg/ml) at 12 hours. Quantitative RT-PCR was performed using premade TaqMan probe for AGT. The quantitative fold changes in mRNA expression were determined as relative to 18S mRNA levels in each corresponding group and calculated using $2\text{-}\Delta\Delta^{CT}$ method. Statistical analysis was undertaken using an independent sample t-test. * = $p<0.05$ was considered as a significant versus untreated cells. * = P value 0.02, ** = p value 0.002 (Overweight); *** = p value <0.001 (Obese ± hypertension).



Adipocyte cells were treated with LPS at 10 ng/ml with and without SN50 (50 μg/ml) at 24 hours. Proteins (20 μg per lane) were separated by 12% SDS-PAGE and analyzed by western blotting using anti- TNF-α antibody. Loading equality was controlled using antibody against β-actin protein.



Adipocyte cells were treated with LPS at 10 ng/ml without and with SN50 (50 μg/ml) at 24 hours. Proteins (20 μg per lane) were separated by 12% SDS-PAGE and analyzed by western blotting using an anti- AGT antibody. Loading equality was controlled using antibody against β-actin protein.



Adipocyte cells were treated with LPS at 10 ng/ml with and without SN50 (50 μg/ml) at different time points. Proteins (20 μg per lane) were separated by 12% SDS-PAGE and analyzed by western blotting using an anti- NF-κB p65 antibody. Loading equality was controlled using antibody against β-actin protein.

BIBLIOGRAPHY

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