Diet and Endoplasmic Reticulum Stress on the Structure-Function of Relationship of the Kidney

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ABSTRACT

Diet is a leading risk factor for cardiovascular disease; however, the underlying mechanisms remain unclear. The kidney plays a significant role in CV function, and endoplasmic reticulum stress (ERS) has been implicated in kidney dysfunction. Therefore, the current study aims to determine if diet influences the severity of kidney dysfunction and ERS indicated by tunicamycin injection. Weanling male C57Bl/6 mice (N=24) were randomly assigned (N=8) to receive a standard chow (Control), a commercial WD (high-fat and high-sugar), or a novel rodent formulation incorporating nutrition observed in Americans called the American Diet (AD). Mice were given ad libitum access to their assigned diets for six weeks. After six weeks, mice from each diet group (N=3-4) underwent a single i.p. injection of a control solution of 1µg/kg body weight of Tunicamycin, an antibiotic known to induce ERS. After 24 hours, all mice fasted for 6 hours before anesthesia (isoflurane), and blood was collected through the retro-orbital sinus. Mice were euthanized, and kidneys were collected. The plasma concentrations of insulin, creatinine, and blood urea nitrogen (BUN) were determined using commercial kits. The mRNA expression of the leukocyte marker CD45+ was quantified in kidney tissue using qPCR. All data were analyzed using General Linear Model Procedures (ANOVA) using SPSS (IBM), and values were considered statistically significant if P<0.05. Diet significantly influences circulating insulin (P=0.017), and creatinine (P=0.005), with mice fed the WD having the highest circulating insulin and mice fed AD having the most elevated circulating creatinine. Mice treated with Tunicamycin had significant reductions in insulin (P=0.008) and BUN (P<0.001), with no significant influence on creatinine or gene expression. Adequate diet and treatment effects were observed for BUN (P=0.001) between groups. Our data suggest that diet significantly influences kidney health, and ERS may mediate this effect.

INTRODUCTION

Diet is a leading risk factor for cardiovascular disease and a primary risk for other conditions such as diabetes, obesity, and cancer. Although having a bad diet can lead to detrimental effects, the underlying mechanisms of different diets remain unclear. The kidneys play a role in CV function, and endoplasmic reticulum stress (ERS) has been implicated in kidney dysfunction. Circulating levels of BUN (Blood Urea Nitrogen) and Creatinine in plasma tested kidney function. BUN outlines the amount of Urea Nitrogen in the blood; a high accumulation in the blood would signify some damage. High creatinine would suggest that the kidney is not filtering blood at total capacity, which can lead to various kidney diseases. Circulating levels of insulin in plasma were also measured to observe the amount of blood sugar regulated by the pancreas and the kidney. Therefore, this study aims to determine if diet influences the severity of kidney dysfunction and ERS indicated by a tunicamycin injection. Tunicamycin is an antibiotic that inhibits the first step in synthesizing N-linked glycans in proteins, causing an accumulation of unfolded glycoproteins in the ER, leading to ER stress (1). This antibiotic was used for the treatment of certain cancers in the prostate and colon.

METHODS

Weanling male C57Bl/6 mice (N=24) were randomly assigned (N=8) to receive a standard chow (Control), a commercial WD (high-fat and high-sugar), or a novel rodent formulation incorporating nutrition observed in Americans called the American Diet (AD). Mice were given ad libitum access to their assigned diets for six weeks. After six weeks, mice from each diet group (N=3-4) underwent a single i.p. injection of a control solution of 1µg/kg body weight of Tunicamycin, an antibiotic known to induce ER stress. After 24 hours, all mice fasted for 6 hours before anesthesia (isoflurane), and blood was collected through capillaries behind the eye socket. Mice were euthanized, and kidneys were collected. The plasma concentrations of insulin, creatinine, and blood urea nitrogen (BUN) were determined using commercial kits. The mRNA expression of the leukocyte marker CD45+ was quantified in kidney tissue using qPCR. Summarize in Figure 1. All data were analyzed using General Linear Model Procedures (ANOVA) using SPSS (IBM), and values were considered statistically significant if P<0.05.

Figure 1. Experimental Approach

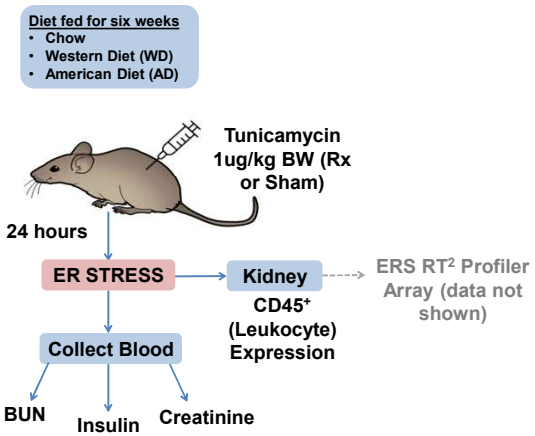


Figure 2. Circulating Creatinine

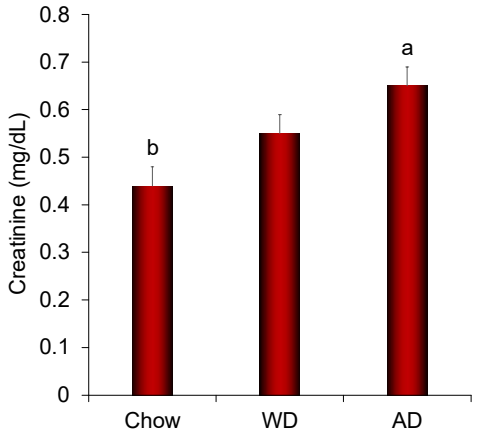


Figure 3. Circulating Insulin

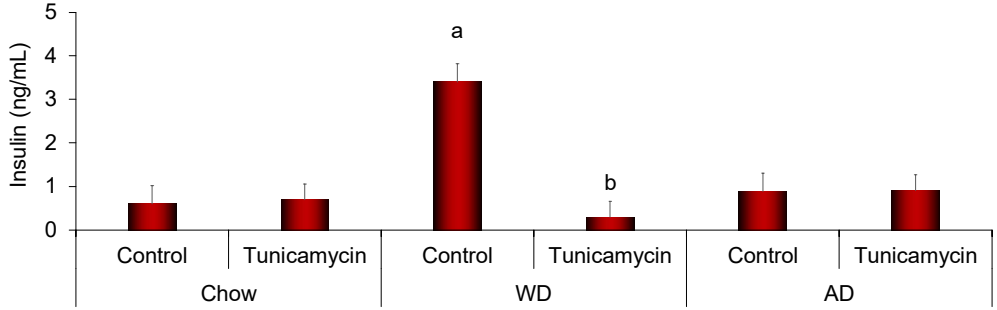
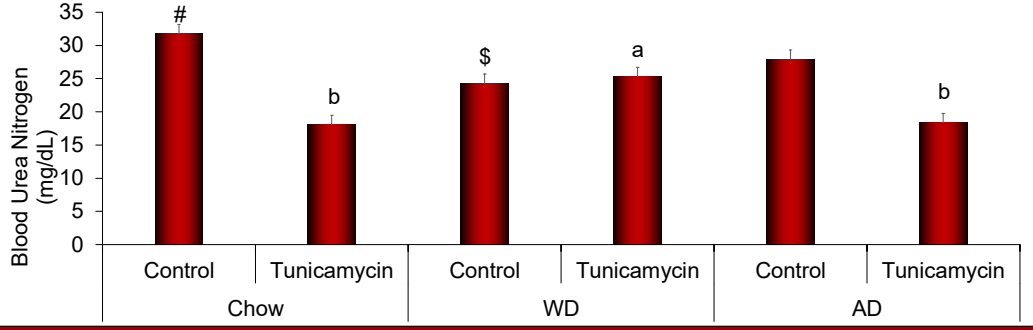


Figure 4. Circulating Blood Urea Nitrogen (BUN)



RESULTS

Figure 2. Mice fed AD diet displayed elevated circulating levels of creatinine in plasma compared to Chow-fed mice (*P=0.005*). Tunicamycin treatments had no observed effect on creatinine levels compared to sham groups and across all three diets (not shown).

Figure 3. Circulating levels in plasma insulin increased significantly (*P>0.001*) in mice fed WD and were unchanged for other diets. Tunicamycin treatment ablated circulating levels of insulin when compared to WD control mice (*P=0.001*).

Figure 4. BUN levels show the amount of urea nitrogen in the blood, which is used to assess kidney function. The graph signifies the statistical difference (*P>0.001*) between the treatment group of the WD (**a**) compared to the Chow (**b**) and AD (**b**) groups. A significant diet effect on BUN was observed between Chow (**#**) and WD (**$**) control groups (*P=0.001*).

CONCLUSION

Studies have shown that obesity causes endoplasmic reticulum (ER) stress, and genes deficient in modulating the stress response, develop insulin resistance linking the progression of type 2 diabetes to insulin receptor signaling *(2)*.

Using a dietary mouse model of obesity, we demonstrate that mice fed different diets for six weeks have a differential response to circulating plasma levels of insulin, creatinine, and BUN when challenged with Tunicamycin, known for inducing ER stress. The expression of CD45+ leukocytes remained unchanged in the kidney, suggesting diet or treatment did not alter leukocyte recruitment.

FUTURE WORK

- We plan to extend our diet treatment beyond six weeks to understand if a prolonged diet further exacerbates kidney or liver dysfunction under ER stress.
- While our previous study was found to induce ER stress using a singular injection, we plan on modifying treatment doses (µg/kg) and times (<>24 hours) in future studies.
- Our group plans to validate our observed expression changes through array studies and western blot analysis.

REFERENCES

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