

# Preventative Effects of Broccoli on Liver Cancer in Young Male Mice

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## Abstract

Liver cancer is a serious disease with a high fatality rate, so preventing it in the first place is key. Environmental toxins and other substances known as xenobiotics which are not a normal part of the diet can contribute to oxidation and liver damage, leading to cancer. In order to prevent this damage from occurring, the human body uses enzymes to neutralize the toxins. One such enzyme is NQO1 (NAD(P)H:Quinone Oxidoreductase-1). In contrast, certain enzymes like CYP2E1 (cytochrome P450 2E1) function in the metabolism and detoxification of many of these chemicals, pre-toxins, and alcohol. During metabolism, CYP2E1 can oxidize some carcinogens into a dangerous, free radical form, which will cause damage if not degraded quickly enough. High levels of CYP2E1 expression have been associated with non-alcoholic fatty liver disease and insulin resistance. Studies have shown that consuming broccoli can have a beneficial effect on NQO1 levels, but consuming a "Western" diet high in fat and sugar can raise CYP2E1 expression. **Our objective was to quantify the gene expression for these two enzymes in young mice (8 and 12 weeks old) on three different diets: control, Western, and Western plus broccoli.** The mice were all treated with a carcinogen (diethylnitrosamine) prior to beginning the diets. Our results indicate that broccoli does have the effect of increasing expression of the NQO1 gene in this study, but there was little impact on expression of the CYP2E1 gene.

## Introduction

According to the American Cancer Society, over 700,000 people are diagnosed with liver cancer every year, and more than 600,000 die. It has been established that diet can impact cancer development, and thus be used as a preventative measure. Diets high in fat and sugar may increase likelihood of developing cancer. Broccoli, with its bioactive component sulforaphane, has been shown to decrease the risk for cancer. Our study was designed to examine whether these effects could be seen in the gene expression of young mice who had been treated with a liver carcinogen but were not yet displaying signs of tumors.

## Design

Our study used 42 male mice, treated with the carcinogen diethylnitrosamine at two weeks of age. Groups are demonstrated in Figure 1. We considered expression of the NQO1 and CYP2E1 genes in the liver within each age group using the control diet as the standard.

## Hypothesis

**Addition of broccoli to the diet of male mice treated with a liver carcinogen will increase expression of the NQO1 gene, an indicator of protection against cancer development. Consumption of a high-fat, high-sugar diet will increase expression of the CYP2E1 gene, a predictor of liver damage.**

## Diet Formulae

	Control (4.00 kcal/g)		Western (4.60 kcal/g)		Western + Broccoli (4.70 kcal/g)	
	g%	kcal%	g%	kcal%	g%	kcal%
Protein	20.30	20.30	23.33	20.30	23.33	20.30
Carbohydrate	63.95	63.95	46.61	40.55	46.61	40.55
Fat	7.00	15.75	20.00	39.15	20.00	39.15
[Broccoli]	-	-	-	-	10.00	-
<b>Total</b>	<b>91.25</b>	<b>100.00</b>	<b>89.94</b>	<b>100.00</b>	<b>99.94</b>	<b>100.00</b>

## Methods

After the specified time on the diet (either 4 weeks or 8 weeks), the mice were killed and their livers harvested. The livers were put into RNAlater solution and frozen at -80° C. RNA extraction was performed using the EZNA Total RNA kit II, with the exception that Trizol was substituted for the RNA-Solv Reagent in the homogenization step. The protocol to recover a total RNA solution is demonstrated in Figure 2. This solution was checked for concentration by spectrophotometric absorption at a wavelength of 260nm, with checks of the 260/280 and 260/230 ratios to assess purity. RNA was then diluted to 200ng/μl and checked for integrity by agarose bleach gel electrophoresis. Samples passing these tests underwent reverse transcription to yield cDNA. Gene expression was measured by real-time qPCR on the cDNA samples. Data are shown as mean ± SD.

Figure 1: Group Design



## Results

The results of gene expression are shown in Figures 3 and 4. The control diet of each age group was used as the standard to compare expression in the other diets. For NQO1, our hypothesis only compared Western + Broccoli diet to Control diet. While data for the Western diet was included in the chart, it was not significantly different from the Control diet (P < 0.05). Likewise, we were primarily interested in the difference between Control and Western diets for the CYP2E1 gene, but the diets had no effect on CYP2E1.

**The results support our hypothesis that dietary broccoli increases expression of the NQO1 gene. However, expression of the CYP2E1 gene did not appear to be affected by the Western diet.**

## Figure 2: RNA Extraction Protocol

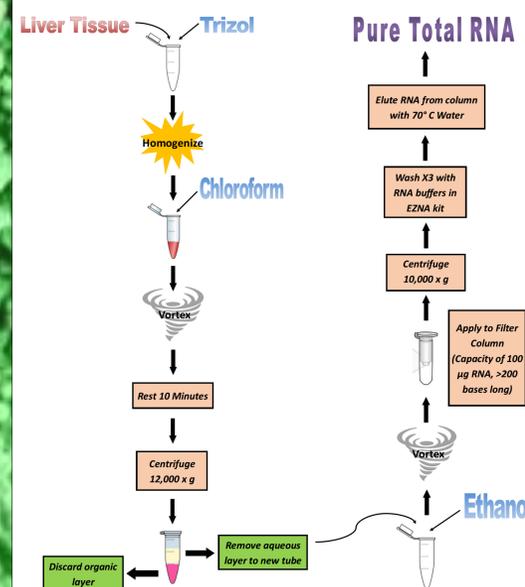


Figure 3a: Broccoli Increases Expression of the NQO1 gene in the Four Week Group

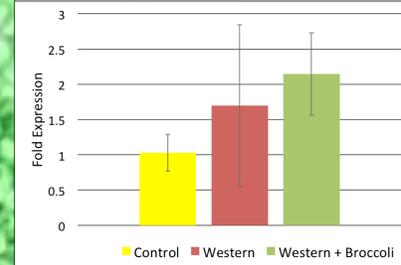


Figure 3b: Broccoli Increases Expression of the NQO1 gene in the Eight Week Group

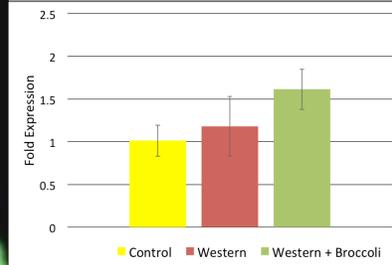


Figure 4a: Western Diet Does Not Affect Expression of the CYP2E1 gene in the Four Week Group

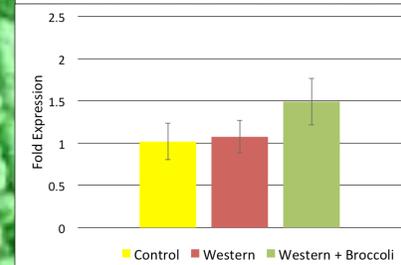
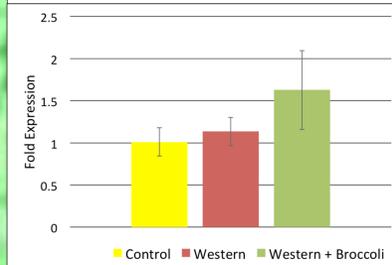


Figure 4b: Western Diet Does Not Affect Expression of the CYP2E1 gene in the Eight Week Group



No Damaging Effect on Liver



## Further Research

This study used a very small sample of mice, so increasing the number would make the data more reliable. It would also be useful to compare a control diet plus broccoli to the Western diet plus broccoli. Further research could be done to investigate gene expression in older mice, or to investigate gene expression in mice who had not been treated with the carcinogen.

## Acknowledgments

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## Conclusions

In mice this young, the Western diet does not appear to impact liver expression of the CYP2E1 gene, leading us to conclude that the diet has not yet had a detrimental effect on liver health that could lead to cancer. Addition of broccoli to the diet did impact liver expression of the NQO1 gene.

**Even short-term intervention with broccoli may have a role in protecting the liver from toxin-induced damage and cancer.**