

Lincoln University

Annual Progress Report: 2012 Formula Grant

Reporting Period

July 1, 2013 – June 30, 2014

Formula Grant Overview

Lincoln University received \$44,916 in formula funds for the grant award period January 1, 2013 through December 31, 2014. Accomplishments for the reporting period are described below.

Research Project 1: Project Title and Purpose

Association of GST Deletions with ITC Metabolism and ROS in African American Students – The main purpose of this preliminary study is to investigate the relationship among glutathione-S-transferase genotype, isothiocyanate metabolism and urinary reactive oxygen species in African American college students. The results will illuminate health disparities in the areas of gene-nutrient interaction and will contribute new knowledge to a field in which the African American population is understudied and therefore likely underserved. Other purposes are to foster collaboration between biology faculty members with expertise in genetics and neuroscience and to establish genotyping and oxidative stress protocols at Lincoln University. In addition, this study will generate preliminary data for publications and for future grant applications.

Anticipated Duration of Project

1/1/2013 – 12/31/2014

Project Overview

High intakes of cruciferous vegetables, such as broccoli, collard greens, cabbage, cauliflower, and mustards, are associated with lower risk of several types of cancer. Isothiocyanates (ITCs) that result from the hydrolysis of glucosinolates found in cruciferous vegetables possess the chemopreventive properties. ITCs induce cell cycle arrest and apoptotic cell death, which can be mediated by reactive oxygen species (ROS). However, glutathione-S transferase (GST) promotes metabolism and elimination of ITCs. There is evidence that GST deletion genotypes lead to slower ITC metabolism, thereby increasing circulating ITCs and lowering cancer risk. Our objective is to delineate the relationship of GST deletion genotypes, ITC metabolism, and ROS in African Americans, a population disparate in cancer outcomes. The GSTM1 genotype is deleted in 16-36% of African Americans, but less data for GSTT1 are available. We hypothesize that GSTM1 and GSTT1 deletion genotypes will be associated with decreased urinary ITC levels and increased ROS in the samples after cruciferous vegetable consumption. Our specific aims are: 1) determine the GSTM1 and GSTT1 deletion frequencies in 50 African American DNA samples purchased from Coriell Institute for Medical Research and in 60 DNA samples collected

from African American college students; 2) determine the relationship between *GSTM1* and *GSTT1* genotypes and urinary ITC levels associated with cruciferous vegetable consumption in 60 African American students; and 3) determine how genetic differences of null GST genotypes and ITC metabolism relate to ROS and glutathione levels in the urine and saliva samples of the African American student population before and after cruciferous vegetable consumption. To accomplish our aims, we will extract DNA from saliva of the African American students and perform multiplex genotyping to determine and confirm published *GSTM1* and *GSTT1* deletion frequencies. We will measure metabolic rates of urinary ITC to determine their relationship with the GST deletions. We will measure ROS and glutathione levels using assays and study gene expression of oxidative stress and apoptosis biomarkers from urine and/or saliva to determine how ROS activity is related to genetic variations in GSTs and ITC levels.

Principal Investigator

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Other Participating Researchers

Karen A. Baskerville, PhD – employed by Lincoln University

Expected Research Outcomes and Benefits

We expect that the *GSTM1* and *GSTT1* null alleles in the African American samples will have decreased urinary isothiocyanate (ITC) levels compared to the wild type GST alleles. We also predict higher levels of reactive oxygen species (ROS) and lower levels of the antioxidant glutathione in response to increased levels of circulating ITCs. There may be differences in the levels of ROS and glutathione in the samples with different null GST genotypes. Higher amounts of both circulating ITCs and ROS have a benefit of decreasing cancer risk as ITCs have been shown to induce apoptosis in cancer. Furthermore, there is very little data in the literature on ROS and glutathione levels after cruciferous vegetable consumption in African American populations. This study will add to the knowledge of *GSTM1* and *GSTT1* deletion frequency in the African American population and the relationship between both ROS and GST deletion genotypes and ITC and GST deletion genotypes in African Americans. In addition, the study will contribute new knowledge about the relationship between ROS and ITC exposure and metabolic rate in African Americans. Improved understanding of ITC-metabolism as well as the production of ROS in response to ITC consumption in combination with GST genotype in an underserved population may be used to inform future dietary guidelines for the consumption of cruciferous vegetables in this population.

Summary of Research Completed

*Aim 1) Determine the *GSTM1* and *GSTT1* deletion frequencies in 50 African American DNA*

samples purchased from Coriell Institute for Medical Research and in 60 DNA samples collected from African American college students.

The copy number assay was established using DNA samples from Coriell Institute for Medical Research and the 7500 Real Time PCR system and the CopyCaller software, both from Life Technology; however, because of the price of the real-time PCR reagents, it was too prohibitive to genotype all the purchased samples. Thus, once the assay was established using the Coriell samples, the DNA samples from the population study at Lincoln University were processed. DNA has been isolated from all 53 saliva samples collected at Lincoln University. DNA concentrations are given in Table 1. All 53 DNA samples have been analyzed for *GSTM1* and *GSTT1* copy number (Table 1). Of these samples, 2 samples contain three copies of the *GSTM1* gene, 11 have two copies, 23 have one copy, and 15 have no copies of the *GSTM1* gene. Only two samples could not be definitively determined for *GSTM1* although it was clear that these two samples contain either one or two copies of *GSTM1*. One sample has three copies of *GSTT1*, 17 have two copies, 24 have one copy, and 11 have no copies of the *GSTT1* gene. Only 3 samples completely lacked both *GSTM1* and *GSTT1*.

Aim 2) Determine the relationship between GSTM1 and GSTT1 genotypes and urinary ITC levels associated with cruciferous vegetable consumption in 60 African American students.

In order to carry out this comparison, we first needed to analyze all urine samples for ITC content. This was a student project that involved several undergraduate students who gained valuable experience in assay preparation and high pressure liquid chromatography using the Waters, 2695 model HPLC Module equipped with a 2996 Photodiode Array Detector. Chromatographic separations was completed using an Agilent Zorbax (SBC18) HPLC column (150mm x 4.6 mm, 3.5 μ m). To date, a total of 25 participants have had all their urine samples analyzed for ITC levels. The number of samples per individual participant ranges from one to five. Out of the samples from 25 participants, 24 gave useable data. We anticipate analyzing another seven samples before the end of August 2014. Several samples were lost in a major power outage in the science building in November of 2013 when the back-up generators failed, thus, the total number of individuals for whom samples are available is 31 rather than the 53 for whom we have DNA. Average ITC concentrations for all analyzed samples are given in Table 2. Initial analysis shows high inter-individual variation in ITC excretion but indicates that, on average, ITC levels peak in urine at 15-18 hours after broccoli consumption; the median peak occurs at 16 hours. Statistical analysis of the relationship between urinary excretion of ITC and *GSTT1* and *GSTM1* genotypes will be conducted after the ITC concentration of all the urine samples has been determined. However, initial analysis does not reveal any relationship between the total copy number of *GSTM1* and *GSTT1* and the rate of urinary ITC excretion.

Aim 3) Determine how genetic differences of null GST genotypes and ITC metabolism relate to ROS and glutathione levels in the urine and saliva samples of the African American student population before and after cruciferous vegetable consumption

ROS in saliva samples was determined using the OxiSelect In Vitro ROS/RNS Assay kit from Cell Biolabs, Inc. To date, all 53 saliva samples have been analyzed in duplicate for ROS.

Table 1: DNA concentrations and genotyping results for *GSTT1* and *GSTM1* from 53 saliva samples.

Sample name	DNA concentration (ug/ml)	<i>GSTM1</i> Copy number	<i>GSTT1</i> Copy number
1000	10	1	1
1001	200	2	1
1002	20	1	0
1003	100	3	0
1003.2	100	1	0
1010	40	1	2
1011	5	2	1
1012	40	3	0
1013	20	1	1
1020	30	1	1
1020.2	15	1	2
2001	40	1	1
2002	100	2	1
2003	15	1	2
2004	100	0	2
2005	10	1	1
2006	10	0	0
2007	20	1	2
2008	15	0	2
2009	10	1	1
2010	50	1	2
2011	30	2	2
2012	25	1	2
2013	40	0	1
2014	50	1	0
2014.2	40	2	2
2015	20	1	1
2016	200	0	2
2018	15	2	2
2019	100	1	2
2020	50	1	1
3001	25	1	1
3002	30	0	1
3003	50	2	1
3004	40	N/A	0
3005	80	2	2
3006	40	0	0
3007	20	1	1
3008	100	2	1
3009	40	1	1
3010	5	1	1
3011	10	0	1
3012	60	0	2
3013	10	0	2
3014	40	0	3
3015	50	1	0
3016	60	0	1
3017	100	0	0
4001	50	2	2
4002	100	2	1
4003	200	0	1
4004	100	0	1
4005	15	N/A	0

Table 2: Isothiocyante concentration in urine samples for all analyzed sample IDs hours after broccoli consumption

ID	Urinary ITC (uM)																			
	Hrs post broccoli: ->	0	2	4	6	8	10	12	14	16	18	20	22	24	26	28	30	32	34	36
1000							2.0	1.2		3.0										
1001		3.3																		
1011								10.7												
1012	1.9											2.2	2.7							
1013								13.6											7.7	0.9
2001			0.0					6.4				1.0								
2002				3.0	0.6	1.9	3.5													
2005				16.5						5.4		2.6								
2006		0.0						0.0	0.0	0.0										
2010								20.1		14.0	44.6		19.1							
2011											1.9		9.3			5.8				
2012				1.1					7.9		2.1									
2014												0.9								
2018										4.0		20.0							5.4	
3001				1.6							5.5								0.7	
3002									9.1			1.8		3.3	3.1	3.1				
3003								15.4				4.5		1.2	1.1					
3005								7.7			10.1	5.1		2.4	1.4					
3008									6.1	1.4									1.0	
3011									3.4	0.0				0.5						
3012															13.0					
3013									12.1	54.0	20.1									
3015									1.6	10.7	3.9	5.6								
3017										33.6	36.2	24.1	1.8							
Average	0.9	3.3	0.0	5.6	0.6	1.9	8.7	5.7	12.6	13.0	10.2	2.0	5.5	4.6	4.5	2.3			7.7	0.9