

**Changes in Pancreatic and Jejunal Histopathology and Serum  
IFN- $\gamma$ , TNF- $\alpha$  Levels in Type 1 Diabetes: Role of Chloroform  
Methanol Soluble Gliadin Proteins.**

**By**

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**Keyword:** Type 1 diabetes; Gliadin specific sphingolipid enriched extract (GSLEE); Jejunal  
hisopathology; Serum cytokines; Immunohistochemistry; ELISA; BBdp rats.

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**(Abstract)**

Chloroform methanol (CM) soluble extract of a cereal-based diet has been suggested to induce type 1 diabetes in an experimental animal model of type 1 diabetes. However, the individual constituent of this extract responsible for the disease induction and its disease pathogenesis mechanism remained unexplored. A previous study in our laboratory failed to show that the sphingolipid enriched fraction of CM soluble extract of wheat gluten triggers type 1 diabetes. Therefore, to study the involvement of CM soluble gliadin proteins in type 1 diabetes, we retrospectively analyzed proteins from sphingolipid enriched extract. SDS-PAGE analysis of CM soluble extract of wheat gluten and sphingolipid enriched fraction exhibited protein bands corresponding to the masses of the wheat gliadins, suggesting the presence of gliadin proteins in the CM soluble sphingolipid enriched extract.

We studied the effect of five different dietary treatments on the histopathology of pancreatic tissue from BBdp rats includes insulinitis scores i.e. lymphocytic infiltration in islet of Langerhans in order to test gliadin specific sphingolipid enriched extract (GSLEE) as possible a diabetogen. However, there

were no significant differences in pancreatic insulinitis scores and lymphoid tissue content due to addition of (GSLEE) to the hydrolyzed casein (HC) diet. We also investigated changes in jejunal histopathology and sera IFN- $\gamma$ , TNF- $\alpha$  cytokine concentration in type 1 diabetes, induced by GSLEE. A decrease in jejunal CD4+ and  $\gamma\delta$  TCR + cell counts and inflammatory cell infiltrate were observed due to presence of CM soluble GSLEE in the HC diet, although this decrease was not statistically significant. A significant increase in sera IFN- $\gamma$  cytokine concentration was found in BBdp rats fed the HC + GSLEE diet as compared to rats on HC diet. A numerical decrease in sera TNF- $\alpha$  concentration was also observed in BBdp rats fed the HC + GSLEE diet, when compared to BBdp rats on the HC diet.

In contrast, a significant increase in serum IFN- $\gamma$  concentrations in BBdp rats were observed after removing the CM soluble GSLEE from the wheat gluten based diet (WG) when compared to the WG diet alone. Removing GSLEE from WG diet resulted in insignificant increase in serum TNF- $\alpha$  concentration in BBdp rats when compared to WG dietary treatment group's BBdp rats. However, there were no significant differences in jejunal enteropathy parameters (i.e. lymphocytic infiltration, mucosal thickness, epithelial erosion, jejunal villi flattenings), jejunal CD4+ and jejunal  $\gamma\delta$  TCR+ cell counts; pancreatic insulinitis scores, lymphoid tissue content after removing the CM soluble GSLEE from the WG diet when compared to the WG diet.

Since overall findings regarding the CM soluble GSLEE's potential to induce type 1 diabetes by changing pancreatic and jejunal histopathology and

elevating serum IFN- $\gamma$ , TNF- $\alpha$  cytokine levels largely remained inconclusive, further investigations are warranted regarding immune suppression potential of the CM soluble sphingolipids in type 1 diabetes and the search of diabetogenic agents remaining in the residue after CM extraction.

**Keyword:** Type 1 diabetes; Gliadin specific sphingolipid enriched extract (GSLEE); Jejunal histopathology; Serum cytokines; Immunohistochemistry; ELISA; BBdp rats.

## **Dedication**

I would like to dedicate my M.S. thesis to my parents Mr. Nagoraoji and Mrs. Lakshmi, and my parents-in-law Mr. Prakashrao and Mrs. Sunandatai for their deepest and selfless love.

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## List of Abbreviations

AA	Amino acid
AAs	Amino acids
AIN	American Institute of Nutrition
ANOVA	Analysis of variance
APCs	Antigen presenting cells
BBdp	Biobreeding diabetes prone rats
Bi	Bicarbonate
BLG	Beta lactoglobulin
BSA	Bovine serum albumin
CBD	Cereal based diet
CCR	Chemokine receptor
CD	Cluster of differentiation
CI	Confidence interval
CM	Chloroform methanol

CTL	Cytotoxic T cell line
CVB	Coxsackie virus-B
DNA	Deoxy-ribose nucleic acid
DQ, DR	MHC class II subregions in humans
EGF	Epidermal growth factor
ELISA	Enzyme-linked immunosorbent assay
GAD	Glutamic acid decarboxylase
GALT	Gut associated lymphoid tissue
GIT	Gastrointestinal tract
GSLEE	Gliadin specific sphingolipid enriched extract
HC	Hydrolyzed casein based diet
HCGSL	Hydrolysed casein based diet with gliadin specific sphingolipid enriched extract added to it
HCSL	Hydrolysed casein based diet with sphingolipid enriched extract added to it
H&E	Hematoxylin and eosin

HEV	High endothelial venule
HLA	Human leukocyte antigen
HMW	High molecular weight
HPLC	High performance liquid chromatography
IA-2	Protein tyrosine phosphatase-2
IDDM	Insulin dependent diabetes mellitus
IECs	Intestinal epithelial cells
IFN- $\gamma$	Interferon gamma
Ig	Immunoglobulin
IGF	Insulin growth factor
IHC	Immunohistochemistry
iIEL	Intestinal intraepithelial lymphocyte
iIELs	Intestinal intraepithelial lymphocytes
IL	Interleukin
kD	Kilo-dalton
KGF	Keratinocyte growth factor

LMW	Low molecular weight
LP	Lamina propria
LPLs	Lamina propria lymphocytes
mAb	Monoclonal antibody
MAdCAM-1	Mucosal addressin cell adhesion molecule-1
MHC	Major histocompatibility
MIP	Macrophage inflammatory protein
MLN	Mesenteric lymph node
MLNs	Mesenteric lymph nodes
mRNA	Messenger ribonucleic acid
NIH	National Institute of Health
NOD	Non obese diabetes mice
NTP	National Toxicology Program
OG 96	Old Guilford 96 diet
PBMC	Peripheral blood mononuclear cell
PBS	Phosphate buffer saline

PCR	Polymerase chain reaction
PLN	Peripheral lymph node
PPs	Payer's patches
P/S	Polyunsaturated/saturated
PUFAs	Polyunsaturated fatty acids
RNA	Ribonucleic acid
RP-HPLC	Reverse phase high performance liquid chromatography
RT-PCR	Reverse transcriptase polymerase chain reaction
SAS	Statistical analysis software
SDS-PAGE	Sodium dodecyl sulphate polyacrylamide gel electrophoresis
SFB	Segmented filamentous bacteria
STAT	Signal transducer and activator of transcription
STZ	Streptozotocin
T <sub>c</sub>	Cytotoxic T cells
TCR	T cell receptor
TCRs	T cell receptors

TGF	Transforming growth factor
Th	T helper
TNF- $\alpha$	Tumor necrosis factor-alpha
WG	Wheat gluten based diet
WGGSLF	Wheat gluten based with gliadin specific sphingolipid enriched extract removed from the diet
WGSLF	Wheat gluten based with sphingolipid enriched extract removed from the diet

## List of Original Publications

**This research is based on the following two research publications and one thesis.**

1. Coleman DL, Kuzava JE, Leiter EH. Effect of diet on incidence of diabetes in nonobese diabetic mice. *Diabetes* 1990;39(4):432-436.
2. Rocher A, Soriano F, Molina E, Gonzalez-Limas G, Mendez E.  
Characterization of distinct alpha- and gamma-type gliadins and low molecular weight components from wheat endosperm as coeliac immunoreactive proteins. *Biochim Biophys Acta* 1995;1247(1):143-148.
3. Shi W. Examination of the effects of a sphingolipid -enriched lipid fraction from wheat gluten on the incidence of diabetes in BBdp rats. M.S. Thesis 2003; Human Nutrition, Food and Exercise Department, Virginia Tech, Blacksburg, USA (to be published).