Liver, Cheese, and Diabetes:
The Effect of Cheese on Hepatic Fat

Taylor Huber, Zohre Hashemi, Catherine Chan

Department of Agricultural, Food and Nutritional Science; University of Alberta

Introduction

- Type 2 diabetes (T2D) is a growing health problem in today's society, with the World Health Organization predicting the number of patients to be 366 million by 2030.[1]
- T2D is characterized by chronic hyperglycemia; disturbances in carbohydrate, fat, protein, and glucose metabolism; and disorders of insulin action and secretion. All of these symptoms can create long-term damage to various organs.[2]
- Non-Alcoholic Fatty Liver Disease (NAFLD), caused by excess fat deposition in liver cells, develops in association with T2D. [3]
- Past studies suggest inverse relations between dairy products, including cheese, and the risk of T2D. The high nutritional content of dairy may reduce body fat and insulin resistance[4], but the effect of the fat content of cheese on liver lipid accumulation has not yet been decided.

Purpose: To determine if cheese has any effect in reducing hepatic lipid accumulation in pre-diabetic rats.

Methods

Animal Model and Diets:

Sprague-Dawley rats were randomized into four diet groups. Except for the Low Fat (LF) Control diet group, the rats were fed a High Fat (HF) diet for 4 weeks. At 4 weeks, and for the rest of the study, the remaining rats were fed three experimental diets : HF Control, HF High Cheese (HF-HCH), HF Low Cheese (HF-LCH). All these three diets were isocaloric and matched for macronutrients. HF-HCH had higher percentage of fat from cheese, whereas HF-LCH had less fat from cheese (Table 1). Rats were fed these diets for 8 weeks.

All animal protocols were approved by the Animal Care and Use Committee at the University of Alberta.

	HF Control	HF-High Cheese	HF-Low Cheese	LF Control
Kcal/1000g	4331.53	3936.58	4028.65	3681.18
% Protein	25.22 %	25.08 %	25.05%	25.28 %
% Protein from cheese	0.00 %	7.40 %	8.79%	0.00 %
% Fat	41.65 %	41.76 %	41.75%	12.28 %
% Fat from cheese	0.00 %	20.86 %	15.63%	0.00 %
% Carbohydrate (CHO)	33.13 %	33.17 %	33.22%	62.44 %
% CHO from cheese	0.00 %	0.38 %	0.46%	0.00 %
% Sugar	27.76 %	27.40 %	27.37%	27.84 %
Total weight	997.8	1094.04	1070.01	995.39
Kcal/total recipe	4322	4306.76	4310.71	3664.2

Table 1. *Composition of the experimental diets*

Methods Cont'd

Tissue Collection:

After 8 weeks on specialized diets, rats were weighed and euthanized. Livers were weighed individually and samples collected were snap-frozen in liquid nitrogen then stored at -80 °C.

Oil Red O Staining:

Two frozen liver slides from each group were covered with Oil Red O (ORO) working solution. After incubation and rinsing, these slides were mounted and prepared for analysis. Using the Zeiss AxioCam HRm microscope attached to a Canon Powershot G10, 30 randomly selected pictures of each slide were taken with 20x objective lens magnification.

Figure 1. *ORO staining procedure*

Quantification:

ImageJ software was used to quantify the ORO stained area in each image. Images were thresholded for lipid droplet signal and percentage values were calculated using Microsoft Excel.

Satatistical Analysis:

Data were analyzed through one-way ANOVA, followed by a PostHoc test on GraphPad Prism and differences were considered significant when $P < 0.05$.

Results

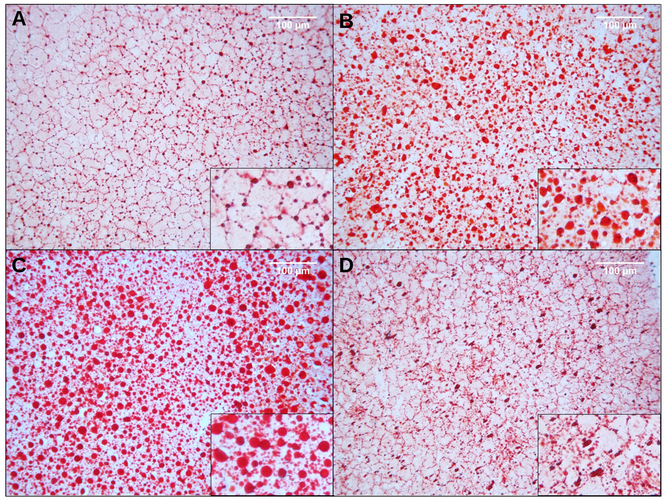

Figure 2. *ORO staining, magnification 20x,*
A: HF Control, B: HF Cheese, C: LF Cheese, D: LF Control,

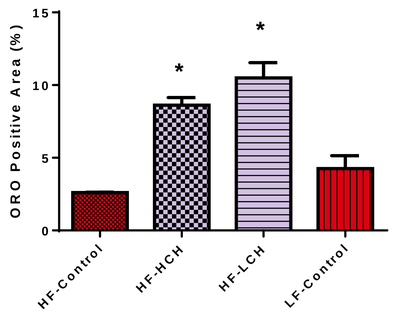

Figure 3. *ORO stained area (%) for four diet groups,*P < 0.05, n = 2 .*

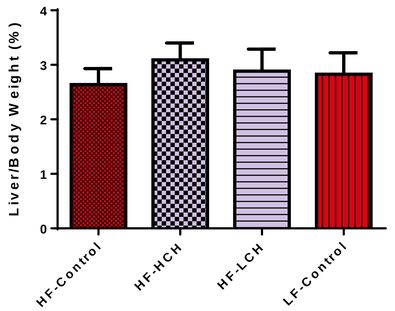

Figure 4. *Weight of liver to total body weight (%), n = 2 .*

Results Cont'd

- ORO stained area was significantly higher in the cheese diet groups compared to the control diet groups. There was no statistical differences between the cheese groups.
- Analysis of liver weight normalized to body weight, as an index of liver lipid content, showed no significant differences between diet groups.

Conclusion

- It appears cheese does not aid in reducing liver fat accumulation; however, it is possible that storing fat in the liver at early stages in disease progression could help with keeping it out of the blood and other organs where it would be more damaging.
- The lack of difference between groups for liver weight could be due to the fact that fat weighs less than protein or carbohydrate, which are the other two major constituents of liver tissue.
- Although results seem conclusive, this study was performed on a small group of samples, and may not be entirely representative of the effects of cheese on lipid accumulation and eventually T2D. More research is needed before the definite effects of cheese can be concluded.

Acknowledgements

Special thanks to everyone in the Chan Lab for answering my questions and for your help in making my summer a fulfilling experience. I would also like to thank my sponsors, Beta Sigma Phi and Canada Summer Jobs, as well as the WISEST team, for making this possible and Jean from the West lab for the use of the microscope. To my teachers, Mme. Daigle and Mr. Gilmour, thank you for your support.

Literature Cited

1. WHO. Definition and Diagnosis of Diabetes Mellitus and Intermediate Hyperglycemia. *Who2*. 2006:50. doi:ISBN 92 4 159493 4.
2. A, Alberti K Zimmet P Ramachandran. Definition, Diagnosis and Classification of Diabetes Mellitus and its Complications Part 1: Diagnosis and Classification of Diabetes Mellitus Provisional Report of a WHO Consultation for the WHO Consultation. (1998): Med15, 539-553.
3. V, Ferramosca A Zara. Modulation of hepatic steatosis by dietary fatty acids. World Journal of Gastroenterology (2014): doi:10.3748/wjg.v20.i7.1746.
4. Aune D, Norat T, Romundstad P, Vatten LJ. Dairy products and the risk of type 2 diabetes: a systematic review and dose-response meta-analysis of cohort studies. *Am J Clin Nutr*. 2013;98(4):1066-1083. doi:10.3945/ajcn.113.059030.