Dietary Lipids Committee Interesterified Fats Workshop

Program & Abstract Book

1 June 2012 **ILSI North America** **Washington DC**

ILSI North America

1156 Fifteenth Street, NW
Suite 200
Washington, DC 20005

1.202.659.0074 voice
1.202.659.3859 fax
www.ilsi.org

Dietary Lipids Committee Interesterified Fats Workshop

June 1, 2012 8:30am-4pm EDT
Location: ILSI NA Offices, Washington, DC

Background and Rationale:

The occurrence of interesterified (IE) fats as a replacement for *trans* fats in the food supply is expanding. While the utility of IE fats for retaining functional properties of food is relatively clear, the nutrition and health implications of this replacement are less well understood. In 2007, Sundram et al.[1] reported that stearic acid at the sn2 position of an IE fat raised LDL:HDL and plasma glucose compared to palm olein. Since this publication, additional clinical trials have examined the effects of short-term consumption of IE fats on postprandial and fasting plasma lipid levels and have not demonstrated similar effects. The impact of long-term consumption (at levels consistent with normal dietary intake and in compositions typically used in food products) on physiological mechanisms and overall diet quality has not been investigated.

In 2011, the Dietary Lipids Technical Committee leadership and the ILSI NA staff conducted a literature search to identify human clinical trials where IE fats were examined. The available literature covered three areas: 1) digestion/absorption; 2) post-prandial effects; and 3) chronic effects.

Therefore, this workshop aims to address these three study areas and has the following **two objectives**:

1. Gain insight into the acute and chronic health impact of IE fats, and

2. Identify key research needs and outline critical considerations for future study designs

PROGRAM

8:30 **Breakfast**

I. Introductions

9:00 **Welcome and Introductions**
Brent Flickinger, ADM and *Eric Hentges, ILSI North America*

Workshop Agenda, Aims and Outcome
Thomas Sanders, King's College London and *Ronald Mensink, Maastricht University*

II. Food science and intake assessment: Replacing *trans* fats and lowering saturated fats in the diet

9:15 **Trends in the interesterification of fats and oils**
Alejandro Marangoni, University of Guelph

9:55 **Estimating potential intakes**
Dave Baer, USDA

III. The impact of IE fats on health - Post-prandial effects

10:35 **Digestion and absorption**
Philip Howles, University of Cincinnati

11:15 **Inflammatory markers, impacts on satiety**
Philip Howles, University of Cincinnati

11:55 **Lunch Break**

III. The impact of IE fats on health - Post-prandial effects (cont'd)

12:40 **Post-prandial effects on hemostatic markers, lipoprotein metabolism and plasma concentrations, glycemic control**
Thomas Sanders, King's College London

IV. The impact of IE fats on health - Chronic/fasting effects

1:20 **Inflammatory and hemostatic markers, impacts on satiety**
Dave Baer, USDA

2:00 **The Longer-Term Effects of Triacylglycerol Structure on Fasting Concentrations of Serum Lipids and Lipoproteins**
Ronald Mensink, Maastricht University

2:40 **Fats with modified triglyceride structure can alter glucose metabolism in humans**
K.C. Hayes, Brandeis University

3:20 **IV. Synthesis and Summary Discussions** - *all speakers*

3:50 **V. Concluding Remarks -** *Thomas Sanders and Ronald Mensink*

SPEAKER BIOS

David J. Baer, PhD, is a Supervisory Research Physiologist with the U.S. Department of Agriculture's Beltsville Human Nutrition Research Center located in Beltsville, Maryland. Dr. Baer conducts controlled dietary intervention studies to investigate the relationship between diet and the risk for chronic degenerative diseases, especially cardiovascular disease, cancer and diabetes in people. His research also includes studies on the health impacts of weight gain and determining the calorie content of foods. Some of the dietary interventions he has investigated include the effects of different types of dairy protein, soy protein, fats and fatty acids, fiber, margarine, butter, plant sterols, salad dressings, nuts, whole grains, berries, alcohol and tea on overall nutrition and health. In addition to dietary intervention studies, Dr. Baer is involved in research studies to validate food survey methodologies and to develop new methods for dietary assessment. Dr. Baer earned his bachelor's degree from the University of Illinois and his doctorate in nutrition from Michigan State University.

Eric J. Hentges, PhD, joined the International Life Science Institute, North America (ILSI NA) as the Executive Director in September 2007. In this capacity he works closely with ILSI NA members, trustees, science advisors, and staff to enhance the organization's programs and the impact of its scientific output. Dr. Hentges joined ILSI NA with over 25 years of experience in nutrition research and education. He has directed strategic research priority planning and administration of competitive research grant programs for several organizations. Additionally, he has directed the development and implementation of nutrition education programs and consumer market research programs. Most recently he served as the Executive Director of the U.S. Department of Agriculture's, Center for Nutrition Policy and Promotion. In this position he had oversight of the USDA's involvement in the development of the 2005 *Dietary Guidelines for Americans* and *MyPyramid, Food Guidance System.* Prior to this, Dr. Hentges served in key positions at the National Pork Board, the National Pork Producers Council, and the National Live Stock and Meat Board. Dr. Hentges holds degrees from Iowa State University, Auburn University and Oklahoma State University. He is a member of the American Society for Nutrition and the Institute of Food Technologists.

Brent D. Flickinger, PhD, is Senior Manager, Nutritional Science for the Archer Daniels Midland Company in Decatur, IL. He has been employed by ADM since April 1999. During his tenure at ADM, his area of expertise and responsibility has grown to include scientific and regulatory support for ADM's entire portfolio of food and dietary supplement ingredients. He and his colleagues evaluate scientific literature to identify new areas for ingredients, conduct evidence-based reviews to substantiate marketing claims, submit product dossiers and rulemaking comments to global regulatory agencies, and provide regulatory guidance for food labeling. Dr. Flickinger received his doctoral degree in Nutritional Sciences from the University of Illinois at Urbana-Champaign and his bachelor's degree in Chemistry from Juniata College in Huntingdon, PA. Immediately prior to joining ADM, he held a postdoctoral research fellowship in the Department of Biochemistry at the University of Texas Health Science Center at San Antonio during which he was awarded an individual NIH Postdoctoral National Research Award fellowship. His training has an emphasis in lipid chemistry, biochemistry and metabolism. He has published in the areas of metabolism of unique dietary fatty acids, cellular targeting of bioactive lipids and emerging research/innovations in dietary fats and oils. Professionally, he is an active member in the American Oil Chemists' Society (past president of the Health and Nutrition Division), the Institute of Food Technologists, the American Society for Nutrition and the American Dietetic Association. Dr. Flickinger also participates in numerous industry associations including Institute of Shortenings and Edible Oils, International Food Information Council (currently co-chair of Dietary Fats Committee), United Soybean Board, International Life Sciences Institute – North America (currently chairman of Technical Committee on Dietary Lipids) as well as the American Heart Association's Industry Nutrition Advisory Panel (currently chair).

K.C. Hayes, PhD, is the author or co-author of more than 200 reports, 24 chapters, and 188 abstracts primarily focused on fats and oils as related to heart disease and diabetes. He obtained his Doctor of Veterinary Medicine from Cornell University and PhD in Nutritional Pathology from University of Connecticut, following which he was a member of the nutrition faculty at the Harvard School Public Health for 15 years before joining the Brandeis faculty as Director of the Foster Biomed Res Lab and university animal facilities. He is a longstanding member of the American Society for Nutrition, including recently being elected a Fellow, and has served on a number of advisory

boards for nutrition, including NIH, USDA and several companies over the years. He is co-inventor on 14 patents, and several discoveries in his lab currently have marketplace applications, eg. identification of a taurine requirement for cats and infant primates led to taurine inclusion in all cat foods and human infant formulas. ALPO cat food originated from research in his lab, and currently most supermarkets in the USA carry SMART BALANCE, a *trans* fat-free margarine designed to improve the LDL/HDL ratio. In addition, recent studies and patents have led to phytosterol-supplemented snack foods that reduce plasma cholesterol, products now available in the US market under the CORAZONAS brand name. Current work in progress (with world patents in place) suggests a promising role for Palm Fruit Juice (oil palm phenolics) as a potent anti-diabetes agent.

Philip N. Howles, PhD, is an Assistant Professor in the Department of Pathology and Laboratory Medicine and the University of Cincinnati College of Medicine in Cincinnati, OH. Dr. Howles has an active research program with a long-standing focus on lipid absorption and lipoprotein metabolism using genetically modified mouse models. His lab was the first to show that Npc1L1 and Zetia reduce fat absorption and weight gain, and improve insulin sensitivity in addition to their ability to block cholesterol absorption, an observation which is now being replicated in some human studies. Understanding diet-gene-drug interactions in this model and how they affect postprandial inflammation, lipoprotein composition and metabolism, as well as hepatic and whole body energy balance is currently a major focus of his lab. Dr. Howles' lab has also established experimental conditions for assaying fasting and postprandial cytokines and leukocytes. He has identified carboxyl ester lipase as a target for raising HDL and increasing reverse cholesterol transport, and has extensive experience with assessing cholesterol and lipoprotein synthesis and flux using a combination of whole animal balance assays, surgical models, and primary hepatocytes. Dr. Howles earned his bachelor's degree from the Cornell University and his doctorate in molecular biology from the State University of New York at Buffalo.

Alejandro G. Marangoni, PhD, is a professor and Tier I Canada Research Chair Food, Health and Aging at the University of Guelph. His work concentrates on the physical properties of foods, particularly fat crystallization and structure. He has published over 200 refereed research articles, nine books, and 14 patents. He is the recipient of many awards including a 1999 Premier's Research Excellence Award, the first Young Scientist Award from the American Oil Chemists' Society (2000), a Tier II Canada Research Chair in Food and Soft Materials Science (2001-2011), two Distinguished Researcher Awards from the Ontario Innovation Trust (2002), a Career Award from the Canadian Foundation for Innovation (2002), an E.W.R. Steacie Memorial Fellowship (2002) – given to the top 6 Canadian scientists from all disciplines - and the T.L. Mounts Award from AOCS in 2004. Dr. Marangoni is a past co-chair of the Natural Sciences and Engineering Research Council of Canada's Plant Biology and Food Science Grant Selection Committee, Editor-in-Chief of Food Research International (Elsevier), an Associate Editor of the Journal of the American Oil Chemists' Society (Springer), and an editorial board member of Food and Function (Royal Society of Chemistry), Food Digestion (Springer) and CYTA-Journal of Food (Taylor and Francis). Dr. Marangoni has co-founded three high-technology companies and is the co-recipient of the 2008 Guelph Partners of Innovation "Innovator of the year" award for his discovery of a platform technology for the manufacture of structured oil-in-water emulsions. Dr. Marangoni is currently Research Program Director for the Ontario Ministry of Agriculture, Food and Rural Affairs' Product Development and Enhancement through Value Chains Program.

Ronald P. Mensink, PhD, received in 1985 an MSc-degree in human nutrition at the Agricultural University in Wageningen. Since 1993 he also has an MSc-degree in epidemiology. At the same university, he received in 1990 his PhD-degree. His thesis dealt on the effects of monounsaturated fatty acids on high-density and low-density lipoprotein cholesterol levels and blood pressure in healthy men and women. In 1990 he moved to the Department of Human Biology at the Maastricht University. Since March 1, 2000 he holds a chair in "Molecular Nutrition." He is leader of Researchline 1 "The Metabolic Syndrome" from NUTRIM (School for Nutrition, Toxicology and Metabolism) and chairman of the Department of Human Biology. His primary research interests are the relationships between nutritive and nonnutritive components in the diet - including functional foods - with risk markers for the metabolic syndrome. Experiments are mainly carried out with human volunteers, but also with transgenic animals and cell cultures. Studies are designed not only to look at effects, but also to unravel the biochemical and molecular mechanisms, which underlie these effects.

Thomas Sanders, BSc PhD DSc, obtained his degrees from the University of London and was appointed Professor of Nutrition and Dietetics at King's College London in 1994 and is currently Head of the Diabetes & Nutritional Sciences Division, School of Medicine, King's College London. His research has focused on the health aspects of dietary fats particularly in relation to cardiovascular disease and type 2 diabetes. He has been principal investigator of a number of large randomized controlled trials that involve lipid manipulation He was a member of the recent WHO/FAO on the role of fats and fatty acids in human nutrition. Much of his early research was involved with omega-3 fatty acid and their role in nutrition. Working with George Miller, he demonstrated some marked effects on the activation of clotting factor VII following meals high in fat. He has conducted a number of studies investigating the effect of fatty acid chain length on haemostatic function and postprandial lipidemia and this led him to investigate the effects of triglyceride structure on these parameters. He has conducted both acute and chronic feeding studies comparing interesterified fats with native fats.

ABSTRACTS

Trends in the interesterification of fats and oils

Alejandro G. Marangoni, PhD

Vegetable oils and fats are the main component of food products such as margarines, shortenings and cooking oils. Many times, however, fats and oil need to be modified in order to achieve a desired functionality.

The physical and chemical properties of fats and oils can be modified by blending, fractionation, hydrogenation and interesterification in order to affect physical properties such as melting and crystallization temperature ranges, solid fat content, solid state structure, and crystal size (1).

Due to health concerns related to high *trans* fatty acid consumption, the food industry has moved away from using partial hydrogenation as the main tool to alter the physical properties of oils to processes such as fractionation and chemical or enzymatic interesterification, as well as genetic modification of the seeds. The interesterification reaction is usually carried out between a high melting fat (fully hydrogenated fat or palm derivatives) and a liquid oil, leading to an exchange of fatty acids within and between triacylglycerols and resulting in the formation of new triacylglycerol molecules with unique properties (desirable plasticity, texture, and mouthfeel) (2). The newly formed molecules have chemical and physical properties in between those of the initial starting materials. This transformation would not be detectable by a common fatty acid analysis; only by a triacylglycerol analysis.

Chemical interesterification usually leads to an increase in the amount of solids at higher temperatures and a decrease in solids at lower temperatures. Such a product would display a wider plasticity range as a function of temperature. Chemical esterification has been used since the late 40's to modify fat properties, as in the case of lard, and has proven itself as a simple, reliable, low cost and reproducible process; however, it has some disadvantages such as high oil losses, low oxidative stability of the finished product, and some flavour reversion problems in the final product (3).

More recently, however, enzymatic interesterification has replaced chemical interesterification as the method of choice to carry out interesterification reactions. Enzymatic interesterification is the main method used for the formulation of low *trans* or *trans* free margarines and shortenings. This lipase-catalyzed interesterification is preferable to chemical interesterification since it does not require the use of chemical catalysts, can be carried out at relatively low temperatures, results in less neutral oil losses, and preserves the oxidative quality of the interesterified oil (4). Drawbacks to enzymatic interesterification include: higher equipment, operating, and catalyst costs, (lipase compared to sodium methoxide in chemical esterification), and usually a more difficult process control.

Enzymatic esterification can be used to make cocoa butter equivalents (CBEs), with a chemical composition that resembles that of cocoa butter more closely than CBEs made using chemical interesterification (5). Another well known use of enzymatic interesterification is for the synthesis of structured lipids with special nutritional or pharmaceutical properties. For example, structured lipids that contain triglycerides with both long-chain, medium- or short-chain fatty acids can be used as human milk fat substitutes, particularly when the positional distribution of long-chain saturates at the sn-2 position in the TAG molecule is preserved (6). Other uses of structured lipids include high-energy drinks for athletes, and for people with an inability to digest triacylglycerols efficiently, as well as for patients experiencing excessive weight loss, and those recovering from illness or injury (5). An interesting use of lipase-catalyzed acidolysis is the enrichment of palm fractions with arachidonic acid, docosahexaenoic acid, eicosapentaenoic acid using a 1,3-specific lipase.

One of the latest developments in the enzymatic modification of lipids is that of structured phenolic lipids synthesis. Structured phenolic lipids are obtained by enzymatic esterification of phenolic acids with triglycerides. These new molecules function very efficiently as lipid-solulble antioxidants (5, 7).

Chemical and enzymatic esterification processes are essential for the modification of the physical properties of oils and fats in the oil processing industry, and are extensively used in the synthesis of triacylglycerols used as human milk fat substitutes, omega-3 enriched triacylglycerols, more digestible fats, confectionery fat substitutes and structured phenolic lipids.

References:

1. O'Brien R (2009) Fats and Oils Processing, Interesterification. In: Fat and oils formulating and processing for applications, CRC Press, 3rd edition, 122-132.
2. Zhang L, Muramoto H, Ueno S, Sato K (2011) Crystallization of fully hydrogenated and interesterified fat and vegetable oil. J. Oleo Sci., 60: 287-292.
3. Rousseau D, Marangoni AG (2002) Chemical Interesterification of Food Lipids: Theory and Practice. In: Food Lipids, Chemistry, Nutrition and Biotechnology. Marcel Dekker, Inc. 2nd edition, 319-352.
4. Gibon V (2011) Enzymatic interesterification of oils. Lipid Technology, 23:274–277.
5. Jala RC, Hu P, Yang T, Jiang Y, Zheng Y, Xu X (2012) Lipases as biocatalysts for the synthesis of structured lipids. Methods Mol. Biol. 861:403-33.
6. Koh SP, Long K, Tan CP et al (2011) The use of enzymatically synthesized medium- and long-chain triacylglycerols (MLCT) oil blends in food application. Int Food Res J 18:355–366.
7. Yang Z, Feddern V, Glasius M, Guo Z, Xu X (2011) Improved enzymatic production of phenolated acylglycerols through alkyl phenolate intermediates. Biotechnol. Lett. 33:673–679.

Estimating Potential Intake of Interesterified Fats

David J. Baer, PhD

National survey data of the intake (as a % of energy) of palmitic and stearic acid suggests a slight increase in both of these saturated fatty acids during the past decade (from 2001 through 2008, the last year national intake data are available)(1-4). Concomitant with the increase in intake of these fatty acids, intake of *trans* fatty acids has decreased (5). Since for some food applications, saturated fats might provide similar functionality as provided from *trans* fatty acids, the increase in intake of palmitic and stearic acids might be associated with the decrease in *trans* fatty acid intake. Moreover, these saturated fatty acids might be from interesterified fats; however, the intake of interesterified fats in the population is largely unknown. One important aspect of estimating intakes of interesterified fats is that it can provide direction for determining how much interesterified fat should be used in clinical trials designed to help unravel the health effects of these fats. In clinical trials, intake of the interesterified fats that exceeds reasonable intake (i.e., mean or 90th percentile of the population) might result in spurious conclusions.

The ILSI Technical Committee on Dietary Lipids completed a modeling exercise to estimate fatty acid intake subsequent to replacement of *trans* fatty-acid containing soybean oil (6,7). An important component of this modeling exercise is that the replacement fats and oils were selected based on their thermal stability or their functional properties. As such, in this modeling exercise, replacement of *trans* fatty acid containing oils in frying processes was with oils with less polyunsaturated fatty acids (i.e., low-linolenic soybean oil, mid-oleic, low-linolenic soybean oil). Replacement of *trans* fatty acid containing oils in foods such as baking, popcorn, shortening, and stick and tub margarines, was with palm-based oils or fully hydrogenated interesterified soybean oil.

In the modeling exercise, 25 food categories were identified which represented 86% of total soybean oil intake and 79% of total *trans* fatty acid intake from NHANES 1999-2002 intake data. Twelve of these 25 food categories were identified as categories for which replacement of *trans* fatty acid containing oils would most likely be replaced with palm oil (some of which may be interesterified) and fully-hydrogenated interesterified soybean oil. *Trans* fatty acid intake from these twelve categories comprised approximately 50% of the total *trans* fatty acid intake, with cakes, cookies, and other baked goods being the predominant dietary source of the *trans* fatty acids.

Based on replacement of palm-based oils or fully hydrogenated interesterified soybean oils in these 12 food categories, predicted intake of palmitic and stearic acids ranges from 1.0 to 2.0% of energy and 0.5 to 1.5% of energy, respectively, for the mean intake of the third quintile, and 1.9 to 4.8% of energy and 09. to 3.8% of energy, respectively, for the mean intake of the 5th quintile. What remains unknown is how much of the total palmitic and stearic acid intake is from interesterified fats. Nevertheless, the upper limit would be 4.8% of energy if all of the palm-oil based products were interesterified.

In five studies (7-12) investigating the effect of interesterified fats on lipids and lipoproteins, the amount of interesterified fatty acid has ranged from 1.9% of energy to 12% of energy (only one study reported using less than 8% of energy). Thus, the amount of interesterified fat in clinical studies appears to be almost twice the amount of interesterified fat that would be consumed at the mean of the 5th quintile of intake. With such amounts, one must consider whether the observed effects are clinically relevant or not.

References

1. U.S. Department of Agriculture, Agricultural Research Service. 2010. Energy Intakes: Percentages of Energy from Protein, Carbohydrate, Fat, and Alcohol, by Gender and Age, What We Eat in America, NHANES 2007-2008. Available: www.ars.usda.gov/ba/bhnrc/fsrg.
2. U.S. Department of Agriculture, Agricultural Research Service. 2008. Nutrient Intakes from Food: Mean Amounts Consumed per Individual, One Day, 2005-2006. Available: www.ars.usda.gov/ba/bhnrc/fsrg.
3. U.S. Department of Agriculture, Agricultural Research Service. 2007. Nutrient Intakes from Food: Mean Amounts Consumed per Individual, One Day, 2003-2004. Available: www.ars.usda.gov/ba/bhnrc/fsrg.
4. U.S. Department of Agriculture, Agricultural Research Service. 2005. Nutrient Intakes from Food: Mean Amounts Consumed per Individual, One Day, 2001-2002. Available: www.ars.usda.gov/ba/bhnrc/fsrg.
5. Doell D, Folmer D, Lee H, Honigfort M, Carberry S. Updated estimate of trans fat intake by the US population. Food Addit Contam Part A Chem Anal Control Expo Risk Assess 2012;29(6):861-74.
6. DiRienzo MA, Astwood JD, Petersen BJ, Smith KM (2006) Effect of substitution of low linolenic acid soybean oil for hydrogenated soybean oil on fatty acid intake. Lipids 41:149–157
7. DiRienzo MA, Lemke SL, Petersen BJ, Smith KM (2008) Effect of substitution of high stearic low linolenic acid soybean oil for hydrogenated soybean oil on fatty acid intake. Lipids 43:451–456.
8. McGandy RB, Hegsted DM, Myers ML. Use of semisynthetic fats in determining effects of specific dietary fatty acids on serum lipids in man. Am J Clin Nutr 1970;23(10):1288-98.
9. Zock PL, Katan MB. Hydrogenation alternatives: effects of trans fatty acids and stearic acid versus linoleic acid on serum lipids and lipoproteins in humans. J Lipid Res 1992;33(3):399-410.
10. Zock PL, de Vries JH, de Fouw NJ, Katan MB. Positional distribution of fatty acids in dietary triglycerides: effects on fasting blood lipoprotein concentrations in humans. Am J Clin Nutr 1995;61(1):48-55.
11. Aro A, Jauhiainen M, Partanen R, Salminen I, Mutanen M. Stearic acid, trans fatty acids, and dairy fat: effects on serum and lipoprotein lipids, apolipoproteins, lipoprotein(a), and lipid transfer proteins in healthy subjects. Am J Clin Nutr 1997;65(5):1419-26.
12. Sundram K, Karupaiah T, Hayes KC. Stearic acid-rich interesterified fat and trans-rich fat raise the LDL/HDL ratio and plasma glucose relative to palm olein in humans. Nutr Metab (Lond) 2007;4:3.

The impact of IE fats on health: Digestion, absorption, and post-prandial inflammation

Philip Howles, PhD

Available Data:

A number of studies have compared the digestion and absorption of fatty acids from a physical mixture of long- and medium- chain triglycerides (TG) versus interesterified (IE) TG derived from the same mixture. While TG digestion is not measurably different between these presentations, the rate and route of absorption of specific fatty acids (FA) can be affected by positon on the glycerol backbone. Long-chain FA (LCFA) are more rapidly transported into lymph from the sn-2 position than the sn-1(3) position (1) although some studies suggest that this

effect varies with dose or time (1,2) and the rate of sn-2 LCFA transport can be reduced by medium-chain FA (MCFA) when the total FA dose is too low to stimulate robust chylomicron synthesis. Data indicate that IE fats provide a mechanism to increase absorption of essential FA in clinical settings such as malabsorption (3,4). Conversely, a greater proportion of MCFA are incorporated into lymph chylomicrons from the sn-1(3) position rather than transported via the portal circulation to the liver as free FA (5,6). Both animal models and clinical studies suggest that IE fats provide a mechanism to deliver MCFA for energy utilization to peripheral tissues in patients with high stress such as burn or surgical trauma, thus diminishing nitrogen wasting and organ damage (7,8). Absorption of fat soluble vitamins (9) and bioavailability of lipophilic drugs (10) is also improved when co-administered with IE fats. Overall FA absorption (24 h) is minimally different between TG mixtures and IE fats of the same composition, except for saturated LCFA (16:0, 18:0) which form calcium soaps and are excreted to a greater degree when in the sn-1(3) position (reviewed in 11). Current data indicate only limited differences in long-term tissue fate of LCFA from mixed TG vs IE fats under normal conditions (12). Incorporation of DHA and EPA into brain phospholipids of rat dams or pups was not affected by dietary lipid structure (13), however EPA (but not DHA) delivery to splenocytes was increased if it replaced all 18:3 FA in the sn-2 position of fed TG (14). Studies of mixed vs. IE TG used in parenteral feeds to dogs revealed no differences in FA tissue fate, however (15). Several studies suggest increased energy expenditure and diminished weight gain without changes in food intake when MCFA is given as IE lipids as compared to mixed TG (e.g. 16). However, reports of satiety and meal pattern studies are limited.

Interest in postprandial inflammation has increased greatly in recent years because of its apparent impact on diabetes and metabolic disease. The results of several studies suggest that dietary FA composition and availability can modulate inflammatory responsiveness, and this area presents opportunities for productive investigation. Although not a postprandial analysis, dietary EPA increased phagocytic capacity of activated monocytes and neutrophils when supplied in the sn-2 position but not in the sn-1,3 position (14), possibly as a result of changes to membrane phospholipids of splenocytes. In an *in vitro* experiment with whole blood from normal individuals, it was found that addition of mixed TG containing MCFA greatly increased activation and degranulation of monocytes and neutrophils as compared to addition of IE TG of similar FA compositon (17). While this study was designed to model effects of parenteral nutrition, it nonetheless underscores the possibility that MCFA have physiologic effects separate from their readily available caloric content. Leukocyte activation markers were also reduced *in vivo* when rats received parenteral MCFA as structured TG vs mixed TG after gastrectomy surgery (18). However, MCP-1 and MIP-2 levels in peritoneal lavage fluid were elevated by the structured TG, leaving the relative risk or benefit open to discussion. In addition to MCFA effects, different availability of saturated LCFA from IE fats vs mixed TG also has the potential to change postprandial inflammation since palmitate and stearate are known to induce various inflammatory responses (as in 19). Data consistent with this notion come from studies of Npc1L1 deficient mice, which have decreased saturated LCFA absorption (20), and which also exhibit reduced postprandial leukocyte activation compared to control mice after a lard gavage (21). Fat absorption has been shown to dramatically increase mucosal mast cell activation with concomitant release of prostaglandin (PGD2) as well as mast cell protease II (22). Importantly, this response was associated only with basolateral chylomicron secretion and was elicited by LCFA but not MCFA. In this report, TG mixtures or IE lipids were not studied, but the results suggest that this would be another area of productive investigation. In addition, studies designed to investigate the links between the post-prandial intestinal inflammatory responses and those described for circulating cytokines and activated leukocytes are warranted, both in general and with specific focus on the type and structure of dietary lipids.

Study Methodology

A large portion of the data centered on digestion and absorption of the various TG mixtures are derived from lymph-cannulated rats or dogs, with radiolabeling and/or mass monitoring used to track the fate of FA from specific positions. Tissue distribution of FA from different TG sources derives largely from composition analysis after animals were given specific diets for several days or weeks. It could be informative to investigate the fate of FA from mixed vs IE TG presentations by more acute methods to determine if there are postprandial differences in tissue targeting and/or metabolism that have not been apparent. Several methods have been used to investigate postprandial inflammation. *In vitro* treatment of blood lekocytes with different lipids may approximate parenteral nutrition effects, but timed measures of leukocyte markers and cytokine levels after lipid gavage is necessary to

accurately assess the transient postprandial effects (23). Gut inflammation and mucosal mast cell activation is measured in lymph cannulated animals, which allows for distinguishing mucosal effects from systemic effects and which can provide more sensitivity because analytes are separated from rapid dilution and clearance by the circulation.

Summary and Conclusions

- Interesterification of TG mixtures containing both LCFA and MCFA provides a mechanism to increase absorption of essential fatty acids, and also increases lymphatic transport of MCFA which can increase delivery of utilizable energy to peripheral tissues. These effects can improve clinical outcomes in cases of malabsorption and in highly stressed patients at risk for tissue wasting and organ failure.
- There is little information about the effects of IE lipids on chylomicron size or apolipoprotein composition. There is also little direct information about acute postprandial tissue fate of lipids from IE vs mixed TG.
- There are no reports of IE TG effects on gut hormones (e.g. GIP, GLP-1) that play roles in insulin sensitivity and metabolic disease.
- Some studies suggest that MCFA may have proinflammatory effects which are ameliorated by interesterification. Further investigation of the effects of mixed vs. IE lipids on postprandial inflammation of the intestine, leukocytes, vascular endothelium, and adipose tissues may be warranted in light of their roles in various metabolic diseases.

References

1. Ikeda I, Tomari Y, Sugano M, Watanabe S, Nagata J (1991) Lymphatic absorption of structured glycerolipids containing medium-chain fatty acids and linoleic acid, and their effect on cholesterol absorption in rats. Lipids 26: 369-373.
2. Couëdelo L, Vaysse C, Vaique E, Guy A, Gosse I, Durand T, Pinet S, Cansell M, Combe N (2012) The fraction of α-linolenic acid present in the sn-2 position of structured triacylglycerols decreases in lymph chylomicrons and plasma triacylglycerols during the course of lipid absorption in rats. J Nutr 142: 70-75
3. Tso P, Lee T, DeMichele SJ (1999) Lymphatic absorption of structured triglycerides v physical mix in a rat model of fat malabsorption. Am J Physiol 277: G333-G340
4. McKenna MC, Hubbard VS, Bieri J (1985) Linoleic acid absorption from lipid supplements in patients with cystic fibrosis with pancreatic insufficiency and in control subjects. J Pediatr Gastroenterol Nutr 4: 45-51
5. Tso P, Karlstad MD, Bistrian BR, DeMichele SJ (1995) Intestinal digestion, absorption, and transport of structured triglycerides and cholesterol in rats. Am J Physiol 268: G568-G577.
6. Mu H, Høy CE. (2002) Distribution of medium-chain FA in different lipid classes after administration of specific structured TAG in rats. Lipids 37: 329-331
7. Teo TC, DeMichele SJ, Selleck KM, Babayan VK, Blackburn GL, Bistrian BR (1989) Administration of structured lipid composed of MCT and fish oil reduces net protein catabolism in enterally fed burned rats. Ann Surg 210: 100-107
8. Kenler AS, Swails WS, Driscoll DF, DeMichele SJ, Daley B, Babineau TJ, Peterson MB, Bistrian BR (1996) Early enteral feeding in postsurgical cancer patients: fish oil structured lipid-based polymeric formula versus a standard polymeric formula. Ann Surg 223: 316-333
9. Tso P, Lee T, DeMichele SJ (2001) Randomized structured triglycerides increase lymphatic absorption of tocopherol and retinol compared with the equivalent physical mixture in a rat model of fat malabsorption. J Nutr 2001;131:2157-2163
10. Holm R, Porter CJ, Edwards GA, Müllertz A, Kristensen HG, Charman WN (2003) Examination of oral absorption and lymphatic transport of halofantrine in a triple-cannulated canine model after administration in self-microemulsifying drug delivery systems (SMEDDS) containing structured triglycerides. Eur J Pharm Sci 20: 91-97
11. Mu H, Porsgaard T (2005) The metabolism of structured triacylglycerols. Prog Lipid Res 44: 430-448
12. Sala-Vila A, Castellote AI, López-Sabater MC (2008) The intramolecular position of docosahexaenoic acid in the triacylglycerol sources used for pediatric nutrition has a minimal effect on its metabolic use. Nutr Res 28: 131-136

13. Sommer-Hartvigsen M, Mu H, Sorig-Hougaard K, Lund SP, Xu X, Hoy CE (2004) Influence of dietary triacylglycerol structure and level of n-3 fatty acids administered during development on brain phospholipids and memory and learning ability of rats. Ann Nutr Metab 48: 16-27
14. Kew S, Gibbons ES, Thies F, McNeill GP, Quinlan PT, Calder PC (2003) The effect of feeding structured triacylglycerols enriched in eicosapentaenoic or docosahexaenoic acids on murine splenocyte fatty acid composition and leucocyte phagocytosis. Br J Nutr 90: 1071-1080
15. Simeons C, Deckelbaum RJ, Carpentier YA (2004) Metabolism of defined structured triglyceride particles compared to mixtures of medium and long chain triglycerides intravenously infused in dogs. Clin Nutr 23: 665-672
16. Kasai M, Nosaka N, Maki H, Negishi S, Aoyama T, Nakamura M, Suzuki Y, Tsuji H, Uto H, Okazaki M, Kondo K (2003) Effect of dietary medium- and long-chain triacylglycerols (MLCT) on accumulation of body fat in healthy humans. Asia Pac J Clin Nutr 12: 151-60
17. Versleijen M, Roelofs H, Preijers F, Roos D, Wanten G (205) Parenteral lipids modulate leukocyte phenotypes in whole blood, depending on their fatty acid composition. Clin Nutr 24: 822-829
18. Lin MT, Yeh SL, Tsou SS, Wang MY, Chen WJ (2009) Effects of parenteral structured lipid emulsion on modulating the inflammatory response in rats undergoing a total gastrectomy. Nutrition 25: 115-121
19. Ajuwon KM, Spurlock ME (2005) Palmitate activates the NF-κB transcription factor and induces IL-6 and TNFα expression in 3T3-L1 adipocytes. J Nutr 135: 1841-1846
20. Labonté ED, Camarota LM, Rojas JC, Jandacek RJ, Gilham DE, Davies JP, Ioannou YA, Tso P, Hui DY, Howles PN (2008) Reduced absorption of saturated fatty acids and resistance to diet-induced obesity and diabetes by ezetimibe-treated and Npc1l1-/- mice. Am J Physiol-Gastrointest Liver Physiol 295: G776-G783
21. Bacon WC, Basford JE, Kuhel DG, Hui DY, Howles PN (2011) Npc1l1 gene ablation reduces diet-induced adipose inflammation in mice. Arterioscler Thromb Vasc Biol 31: (mtg abstract and submitted manuscript)
22. Ji Y, Sakata Y, Yang Q, Li X, Xu M, Yoder S, Langhans W, Tso P (2012) Activation of rat intestinal mucosal mast cells by fat absorption. Am J Physiol-Gastrointest Liver Physiol [March 29 Epub]
23. Gower RM, Wu H, Foster GA, Devaraj S, Jialal I, Ballantyne CM, Knowlton AA, Simon SI (2011) CD11c/CD18 expression is upregulated on blood monocytes during hypertriglyceridemia and enhances adhesion to vascular cell adhesion molecule-1. Arterioscler Thromb Vasc Biol 31: 160-166

Post-prandial effects on hemostatic markers, lipoprotein metabolism and plasma concentrations, glycemic control

Thomas Sanders, BSc PhD DSc

Available data

There are a limited number of studies that have made head to head comparisons between interesterified fats and comparators with a similar fatty acid composition (Berry 2009). Interesterification had been used to compare stearic rich fats with oleic acid rich fats in some earlier studies. These studies employed a blend of fully hydrogenated high oleic acid sunflower oil with native high oleic sunflower oil. These studies found a slower increase in plasma triglycerides and a lower area under the curve (Sanders *et al.* 2000; Tholstrup *et al.* 2003). Subsequent studies (Sanders *et al.* 2001) comparing a structured triglyceride (SALATRIM) rich in stearic acid found markedly depressed postprandial increases in plasma triglycerides compared with high oleic sunflower oil or coco butter. The depressed postprandial lipemia was accompanied by decreased activation of clotting factor VII as measured as factor VII coagulant activity or activated FVII concentration. There were no effects on indices of fibrinolysis (plasminogen activator inhibitor type 1, or tissue plasminogen activator. A follow-up study (Sanders *et al.* 2003) demonstrated that randomization of cocoa butter decreased postprandial lipemia and factor VII activation compared with native cocoa butter. This study also demonstrated that the fatty in the sn-2 position of the triglyceride was retained in that position upon absorption as demonstrated by the analysis of chylomicrons as previous demonstrated by Summers *et al.* (1999).

Berry *et al.* (2007a) made a comparison between native and interesterified shea butter and found that both native and interesterified shea resulted in less postprandial lipemia and decreased factor VII activation compared with high oleic sunflower oil. She postulated that the differences were due to the difference in solid fat content above 37 °C measured by NMR or by differential scanning calorimetry. In a follow-up study, a comparison was made between randomized shea butter and high oleic sunflower oil and this indicated that the shea butter decreased postprandial lipemia but also increased lipoprotein lipase activity. Berry *et al.* (2007b) then compared interesterified with native super palm olein on postprandial on postprandial lipemia and plasma glucose and insulin. Postprandial lipemia was lower on the interesterified fat but there was a tendency for postprandial plasma insulin to be lower and glucose to be higher.

Zampelas *et al.* (1994) compared 40g of palm olein (6% 16:0 at *sn*-2) with Betapol™ (73% 16:0 at *sn*-2) in 16 healthy men but found no differences in postprandial TAG concentrations. However, the level of fat in the test meal was low and the participants were young and healthy. Summers *et al.* (1993)also found no differences in postprandial TAG using similar fats but with 60g fat test meals but only 6 women and 2 men were studied. Yli-Jokipii *et al*. (2001) compared PO (16% 16:0 at *sn*-2) with IPO (42% 16:0 at *sn*-2) in meals providing 55g fat /m^2 body surface area in 10 pre-menopausal women but found no difference in postprandial TAG. Yet the same group (Yli-Jokipii *et al*., 2003) reported higher postprandial TAG following interesterified lard (52% 16:0 at *sn*-2) compared with native lard (68% 16:0 at *sn*-2) in 7 female and 2 male subjects but the iAUC did not achieve statistical significance. However, both these studies were statistically underpowered.

Sanders *et al*. (2011) compared interesterified palm olein (IV 56) with native palm olein, lard and high oleic acid sunflower oil in a two centre study (London and Maastricht) in healthy participants (25 male, 25 females). They found a tendency for the initial increase in triglycerides to be lower following the interesterified fat. There was no evidence to indicate an increased production of apolipoprotein B48 production or resistance times of chylomicron remnants following the interesterified fat. No differences in postprandial glucose, insulin, c-peptide release. However, but found a substantially lower secretion of glucose-dependent insulinotropic polypeptide following the lard and interesterified palm oil compared. They noted substantial gender differences in response to the test meals for plasma TAG, insulin and glucose.

Study methodology

Most studies have used a randomized crossover design using a standardized test meal. Typically a test meal needs to supply 50g fat to induced postprandial lipemia and 75-80g carbohydrate to provide a glycemic challenge. In practical terms it is not possible to extend follow-up beyond 8 h following a test meal because subjects are then a fasting state and this results in significant mobilisation of fat. Most studies, except Sanders. *et al* (2001), were conducted in healthy young subjects. It is noteworthy that young women are far less sensitive to dietary fat than men.

Cannulation is preferred. In our studies we have been careful to fully characterise the test materials used in terms of fatty acid composition, triglyceride structure and physical characteristics (differential scanning calorimetry, solid fats by NMR). Some studies have used test materials that are not comparable to fats in commercial use. Young female subjects are relative insensitive to postprandial challenges of dietary fat.

References

1. Berry SE. Triacylglycerol structure and interesterification of palmitic and stearic acid-rich fats: an overview and implications for cardiovascular disease. Nutr Res Rev 2009;22:3-17.
2. Berry SE, Miller GJ, Sanders TA. The solid fat content of stearic acid-rich fats determines their postprandial effects. Am J Clin Nutr 2007;85:1486-94.
3. Berry SE, Woodward R, Yeoh C, Miller GJ, Sanders TA. Effect of interesterification of palmitic acid-rich triacylglycerol on postprandial lipid and factor VII response. Lipids 2007;42:315-23.
4. Sanders TA, Berry SE, Miller GJ (2003). Influence of triacylglycerol structure on the postprandial response of factor VII to stearic acid-rich fats. Am J Clin Nutr 77: 777-782.

5. Sanders TA, de Grassi T, Miller GJ, Morrissey JH (2000). Influence of fatty acid chain length and cis/trans isomerization on postprandial lipemia and factor VII in healthy subjects (postprandial lipids and factor VII). Atherosclerosis 149: 413-420.
6. Sanders TA, Filippou A, Berry SE, Baumgartner S, Mensink RP (2011). Palmitic acid in the sn-2 position of triacylglycerols acutely influences postprandial lipid metabolism. Am J Clin Nutr 94: 1433-1441.
7. Sanders TA, Oakley FR, Cooper JA, Miller GJ. Influence of a stearic acid-rich structured triacylglycerol on postprandial lipemia, factor VII concentrations, and fibrinolytic activity in healthy subjects. Am J Clin Nutr 2001;73:715-21.
8. Summers LK, Fielding BA, Herd SL, Ilic V, Clark ML, Quinlan PT, Frayn KN. Use of structured triacylglycerols containing predominantly stearic and oleic acids to probe early events in metabolic processing of dietary fat. J Lipid Res 1999;40:1890-8.
9. Teng KT, Nagapan G, Cheng HM, Nesaretnam K. Palm Olein and Olive Oil Cause a Higher Increase in Postprandial Lipemia Compared with Lard but Had No Effect on Plasma Glucose, Insulin and Adipocytokines. Lipids 2011; 46:381-8.
10. Tholstrup T, Miller GJ, Bysted A, Sandstrom B (2003). Effect of individual dietary fatty acids on postprandial activation of blood coagulation factor VII and fibrinolysis in healthy young men. Am J Clin Nutr 77: 1125-1132.
11. Yli-Jokipii K, Kallio H, Schwab U, Mykkanen H, Kurvinen JP, Savolainen MJ, Tahvonen R. Effects of palm oil and transesterified palm oil on chylomicron and VLDL triacylglycerol structures and postprandial lipid response. J Lipid Res 2001;42:1618-25.
12. Yli-Jokipii KM, Schwab US, Tahvonen RL, Kurvinen JP, Mykkanen HM, Kallio HP. Chylomicron and VLDL TAG structures and postprandial lipid response induced by lard and modified lard. Lipids 2003;38:693-703.
13. Zampelas A, Williams CM, Morgan LM, Wright J, Quinlan PT. The effect of triacylglycerol fatty acid positional distribution on postprandial plasma metabolite and hormone responses in normal adult men. Br J Nutr 1994;71:401-10.

The impact of IE fats on health: chronic/fasting effects on inflammatory and hemostatic markers, and impacts on satiety

David J. Baer, PhD

With the increasing opportunity to use interesterified fats in the food supply, there is an inherent need to understand the health effects associated with consumption of these fats in which specific fatty acids are located at specific sites of the glycerol molecule. Most of the research has been focused on postprandial effects of consumption of interesterified fats on lipids and glucose metabolism, or chronic consumption of these fats on lipoprotein profile. A few studies have reported effects on factors associated with hemostasis.

Pubmed and Scopus databases were searched in order to find publications related to interesterified fats and markers of inflammation, hemostasis, coagulation, and satiety. Searches included general terms related to each area (i.e., inflammation) as well as specific terms for that area (i.e., c-reactive protein for inflammation). Reference sections from review papers were evaluated to find additional papers. The search was expanded to include papers related to cocoa butter, specifically. Of interest were publications that included long-term feeding of diets which did not focus on postprandial events. Albeit this search was not as systematic as it could have been, two papers were identified that are related to the topics of interest (1,2). Both of these papers focus on hemostatic parameters. No papers were identified that report on inflammatory markers or on satiety.

Kelly et al. (1) conducted a randomized crossover study with 13 males to determine the effects of diets enriched in either palmitic acid or stearic acid. The stearic acid test fats used to alter fatty composition of the diet were prepared by interesterification of 100% hydrogenated canola oil and high-oleic sunflower oil whereas the palmitic acid test fat was prepared by interesterification of palm stearin, palm olein, and high-oleic sunflower oil. Diets were fed for 4

weeks (30% of energy from fat, ~6.6% of energy as stearic acid in the stearic acid diet and ~7.8% of energy as palmitic acid in the palmitic acid diet). With respect to their findings on hemostatic markers, fibrinogen, plasminogen, antithrombin III, and activated partial thromboplastin time were not altered by diet. Factor VIII (% activity) decreased between the baseline and final day of the stearic acid diet whereas it was not altered during the course of the study when subjects consumed the palmitic acid test diet. There were no differences in FVIII (% activity) between the two treatments at the end of the test period. Prothrombin time increased for both diets between the initial and final day of the study period but were not different between the two diets.

Meijer and Westrate (2) conducted a double-blind, crossover study with 60 people (30 males, 30 females) to determine the effects of random chemical interesterification on blood lipids, enzymes, and hemostasis parameters. A fat blend was prepared using coconut oil, palm oil, dry-fractionated palm oil-stearin fraction, and a low-*trans* fatty acid, partially hydrogenated canola oil. This blend was used both in its native form and chemically interesterified, and each blend was mixed with soybean oil to prepare two different margarines. Both fat blends were fed at two energy levels (4 % and 8% of energy) with a crossover between the two treatments within an energy level for three weeks each. Plasminogen activator-inhibitor-1 antigen (PAI-1 Ag), tissue-plasminogen activator antigen (t-PA Ag), tissue-plasminogen activator (t-PA) activity, von Willebrand Factor (vWF), activated coagulation factor VII (Factor VIIa) and fibrinogen did not differ between the two treatments or energy level of the margarine used. D-dimers were lower after consumption of the interesterified fat.

Of note is the lack of data on markers of inflammation, hemostatic parameters or satiety from studies in which interesterified fats have been fed for several weeks. These data are needed to help understand the broader impact of interesterified fats on health outcomes. Moreover, these data need to be from appropriately designed and controlled studies to minimize extraneous factors.

References

1. Kelly FD, Sinclair AJ, Mann NJ, Turner AH, Abedin L, Li D. A stearic acid-rich diet improves thrombogenic and atherogenic risk factor profiles in healthy males. Eur J Clin Nutr 2001;55(2):88-96.
2. Meijer GW, Weststrate JA. Interesterification of fats in margarine: effect on blood lipids, blood enzymes, and hemostasis parameters. Eur J Clin Nutr 1997;51(8):527-34.

The Longer-Term Effects of Triacylglycerol Structure on Fasting Concentrations of Serum Lipids and Lipoproteins

Ronald P. Mensink, PhD

Interesterified and fully hydrogenated oils may have a higher proportion of saturated fatty acids at *sn*-2 (and consequently a lower proportion of unsaturated fatty acids at *sn*-1,3) than the native oils have. Here, a short overview is given of some human studies, especially designed to study the effects of these fats and oils on fasting serum lipid and lipoprotein concentrations.

Palmitic acid: Zock et al. (1995) found no major differences between effects of palm oil and enzymatically-interesterified palm oil on serum lipid and lipoprotein concentrations. A similar conclusion was drawn by Nestel et al. (1995). Christophe et al. (2000) also reported no effects on the serum lipoprotein profile, when the effects of butter were compared with those of enzymatically-interesterified butter. Based on these three studies, it can be concluded that interesterification of 16:0-rich fats and oils does not adversely change fasting serum lipoprotein concentrations. However, another line of evidence suggests that in natural oils 16:0 is not a cholesterol-raising fatty acid when predominantly attached to the *sn*-1,3 position (Voon et al., 2011; Choudhury et al., 1995; Ng et al., 1992). This hypothesis however is not supported by all studies (Clifton, 2011; Tholstrup et al. 2011).

Stearic acid: Grande et al**.** (1970) found that natural cocoa butter and imitation cocoa butter had similar effects on serum total cholesterol. Berry et al**.** (2007) showed comparable effects of native and interesterified shea butter on

plasma total cholesterol, LDL cholesterol, HDL cholesterol and TAG concentrations.

Interesterified fat blends: Meijer and Weststrate (1997) found no evidence that interesterification of a fat blend consisting of 36% (w/w) coconut fat, 33% palm oil, 22% dry-fractionated palm oil-stearin, and 9% low-*trans* partially hydrogenated rapeseed oil changed serum lipid concentrations. Sundram et al. (2007), however, suggested that 18:0 may not be a neutral saturated fatty acid, at least when randomized or when it becomes a major saturated fatty acid in the diet.

Conclusion: Overall, the majority of studies discussed do not suggest that at current intakes interesterified fats have adverse effects on the fasting serum lipoprotein profile.

References

1. Berry SE, Miller GJ, Sanders TA. The solid fat content of stearic acid-rich fats determines their postprandial effects. Am J Clin Nutr 2007;85: 1486-1494.
2. Choudhury N, Tan L, Truswell AS. Comparison of palm olein and olive oil: effects on plasma lipids and vitamin E in young adults. Am J Clin Nutr 1995;61: 1043-1051.
3. Christophe, AB, De Greyt WF, Delanghe, JR, Huyghebaert, AD. Substituting enzymatically interesterified butter for native butter has no effect on lipemia or lipoproteinemia in man. Ann Nutr Metab 2000;44: 61-67.
4. Clifton PM. Palm oil and LDL cholesterol. Am J Clin Nutr 2011;94: 1392-1393.
5. Grande F, Anderson JT, Keys A. Comparison of effects of palmitic and stearic acids in the diet on serum cholesterol in man. Am J Clin Nutr 1970;23: 1184-1193.
6. Meijer GW, Weststrate JA. Interesterification of fats in margarine: effect on blood lipids, blood enzymes, and hemostasis parameters. Eur J Clin Nutr 1997;51: 527-534.
7. Nestel PJ, Noakes M, Belling GB, McArthur R, Clifton PM. Effect on plasma lipids of interesterifying a mix of edible oils. Am J Clin Nutr 1995;62: 950-955.
8. Ng TK, Hayes KC, DeWitt GF, Jegathesan, M, Satgunasingam, N, Ong AS, Tan D. Dietary palmitic and oleic acids exert similar effects on serum cholesterol and lipoprotein profiles in normocholesterolemic men and women. J Am Coll Nutr 1992;11: 383-390.
9. Sundram K, Karupaiah T, Hayes KC. Stearic acid-rich interesterified fat and trans-rich fat raise the LDL/HDL ratio and plasma glucose relative to palm olein in humans. Nutr Metab (London) 2007;4: 3.
10. Tholstrup T, Hjerpsted J, Raff M. Palm olein increases plasma cholesterol moderately compared with olive oil in healthy individuals. Am J Clin Nutr 2011;94: 1426-1432.
11. Voon PT, Ng TK, Lee VK, Nesaretnam K. Diets high in palmitic acid (16:0), lauric and myristic acids (12:0 + 14:0), or oleic acid (18:1) do not alter postprandial or fasting plasma homocysteine and inflammatory markers in healthy Malaysian adults. Am J Clin Nutr 2011;94: 1451-1457.
12. Zock PL, De Vries JH, De Fouw N, Katan MB. Positional distribution of fatty acids in dietary triglycerides: effects on fasting blood lipoprotein concentrations in humans. Am J Clin Nutr 1995;61: 48-55.

Fats with modified triglyceride structure can alter glucose metabolism in humans

K. C. Hayes, Brandeis University, P Khosla, Wayne State University, K Sundram, Malaysian Palm Oil Council

Consumption of fat that contains triglycerides with altered structure, ie. where triglyceride molecular species (TG-MS) are rearranged from their natural fatty acid placement on glycerol, has been shown to alter cell physiology and energy metabolism unfavorably when consumed in abundance. This is most well documented for adverse effects on lipoprotein metabolism when feeding restructured fats that contain sn2-*trans*, sn2-16:0, or sn2-18:0 (Hayes and Pronczuk, 2010). *Trans* fatty acids are incorporated during partial hydrogenation of vegetable oils when polyunsaturated fatty acids are modified as mono- and di- *trans* fatty acids, ie. sn2-PUFA are reconfigured to form sn2-*trans* 18:1 and sn2-t,t 18:2, among others. In a similar vein, random inter-esterification of naturally saturated

oils, like palm olein or cocoa butter, rearrange their sn1,3-16:0 or sn1,3-18:0, respectively, displacing the usual sn2-18:1 or -18:2, with a saturated 16:0 or 18:0 at sn2.

Both in the case of partial hydrogenation and inter-esterification (IE), these modified TG-MS have been shown to have unfavorable repercussions. As mentioned, most notably on lipoprotein metabolism (Mensink and Katan, 1993; Zock and Katan, 1992) and more recent evidence suggests a negative impact on glucose metabolism, as well (Sundram et al., 2007).

The purpose of this overview is to scrutinize the latter point, including the impact of fatty acids incorporated in IE fats, on glucose/insulin metabolism within the context of type 2 diabetes mellitus (T2DM), identifying differences in study design that might provide clues to the somewhat unique observations for the sn2-SFA perturbation of glucose metabolism. In preface, it is noteworthy that one of the most egregious insults introduced by chronic *trans* fat consumption is its strong association with risk for T2DM (Riserus et al., 2009) and Metabolic Syndrome (Riccardi et al., 2004). Study of acute *trans* fat intake (6 weeks) in patients with T2DM also has been linked to increased insulin secretion postprandially relative to monounsaturated fat intake (Christiansen et al., 1997).

Dietary Fat and glucose metabolism. Review of the literature makes several points. First, the influence of dietary fat on glucose and insulin metabolism is highly affected by the individual under study: healthy subjects tend to be relatively unresponsive to diet challenge, either as fat saturation or TG-MS, while those with hyperlipidemia or T2DM are more likely to be impacted (Riccardi, 2004; Christiansen et al., 1997; Lopez et al., 2011) than healthy individuals (Lovejoy et al., 2002). The point is that energy metabolism under stress (eg. in MetS or T2DM) is responsive to dietary manipulation, whether it be from fat, type and amount of carbohydrate (including fiber), or protein consumed (Nilsson et al., 2004). Age is likely a factor as well, young individuals tending to be minimally responsive compared to older people, who are more likely to be afflicted with weight issues and a T2DM bias. Furthermore, certain genotypes are likely involved, with populations having a “thrift gene" propensity being most responsive, again related to possible T2DM issues (Pavkov, 2007).

Study design is an equally important consideration with single-meal postprandial evaluation much less informative than pre-exposing subjects to the test fat for 2 - 4 weeks, thereby allowing the body fat pool to adjust to diet fat alterations. Ultimately, dietary fat impacts tissue phospholipids, particularly muscle membranes that can affect insulin sensitivity and glucose uptake (Borkman et al., 1993; Riserus et al., 2009), or as cell signals affecting insulin secretion (Lee et al., 2001). Thus, allowing adaptation to a given fat saturation or TG-MS structural alteration is important when attempting to detect their influence. The amount and kind of CHO fed with the fat makes a difference as well (Pedersen et al., 1999), and protein amount and quality accompanying the fat would also seem potentially important, though less studied (Nilsson et al., 2004; Nuttall and Gannon, 2012).

In terms of mechanisms that have the potential to influence several aspects of glucose metabolism, incretin (eg. GIP and GLP-1) synthesis and release by intestinal mucosal cells is strongly influenced by fat, stimulating insulin release during food digestion and absorption (Thomsen et al., 2003). The ability to modify membrane phospholipid sn2-PUFA content has the potential to alter muscle and adipose glucose uptake by changing membrane fluidity, insulin binding, and cell signals that modulate glucose-transporter activity (Borkman et al., 1993). Presumably, incorporating sn2-SFA in membrane PL would not be desirable based on this reasoning (Hayes, 2001), as it could adversely increase insulin resistance of hepatocytes as well as muscle and adipocytes.

An overview of glucose metabolism for possible aspects that would be impacted by dietary fat, would start with the rate of *glucose/energy absorption*, because slowed absorption reduces the acute burden on insulin needed for glucose metabolism. To that point, a second consideration is *insulin secretion* (availability), while a third concept would include *insulin resistance* (ie. factors or mechanisms that impair GLUT 4 and GLUT 2 glucose transporters in muscle /adipose and liver). In essence, for glucose to be metabolized effectively (to prevent diabetes) one needs to ascertain that glucose input (absorption and liver production rate) can be cleared from the blood quickly, which in the first instance means good insulin sensitivity by adipose, muscle, and liver tissue without causing *insulin resistance*; and, if *insulin resistance* develops, to be sure that adequate *production of insulin* by beta cells can be sustained to overcome the *insulin resistance* and maintain low blood glucose. This is critical to prevent the

hyperglycemia and glycosylation of sensitive tissues, including kidney and arterioles in the retina and peripheral tissues,that ensues once impaired metabolism induces chronic hyperglycemia.

Various studies.

Examination of Sundram et al., 2007 for design considerations, including potentially relevant diet x gene interactions raises several issues. For example, the population under study in Malaysia was composed of native Malays, which represent a gene pool from South Asia known to be highly prone to type 2 diabetes (Abdul-Aziz et al., 2011). Presumably this susceptibility reflects the "thrift gene", documented for its propensity to produce a high incidence of diabetes when appropriately challenged by dietary circumstances beyond an ordinary low-density diet (Pavkov et al., 2007).

A second critical aspect of the design was the inter-esterification (IE) with a high level of 18:0 (to provide 12% of calories in final diet) using fatty acids from fully-hydrogenated soybean oil inter-esterified into soybean oil then blended with natural soybean oil to produce the level of dietary 18:0 desired. The IE fat was selected for melt point characteristics needed for final product functionality, coupled with the fact that this level of 18:0 in IE fat was fed previously to humans for comparison with *trans* fatty acids and an 18:2-rich fat (Zock and Katan., 1992).

A third point to consider is the amount of test fat as a percentage of the total daily fat consumed, which was >70% in this study, designed so that the IE fat would actually have a meaningful impact on lipid metabolism. Studies that incorporate low levels of IE test fat, for example below 50% of total fat, are not likely to detect an adverse effect of an IE fat because the body is able to compensate over the short term when only modestly challenged. In essence, one can design the study either to look for an adverse effect of the IE fat, or the IE fat can be diluted by an excess of the dietary fat to make any potential deleterious effect less detectable in terms of the physiological response.

Another critical aspect of detecting a meaningful response is whether the fat was fed for a sufficiently long time prior to assessing metabolic parameters of interest. For example, several studies have attempted to measure an IE fat affect with a single postprandial meal setting, following the dynamics of fat absorption and or glucose and insulin response for only 4h to 8h postprandially, with the assumption that the IE fat, if seriously compromising, will elicit an adverse response in one or more of the parameters measured. However, postprandial studies in themselves are fraught with design problems as to gender selection, the amount and kind of test fat incorporated into a meal, the amount and kind of protein and carbohydrate in the test meal, and the length of time to follow the post-absorptive process. Since each of these factors can have a bearing on the postprandial response parameters, it becomes imperative that design standardization be established if one is to observe an impact of a single fat in a single meal in an individual who has not been previously acclimated to the fat in question.

The importance of allowing time for adaptation to the test fat is apparent in the designs of Christensen et al., 1997 and Sundram et al., 2007, which both included midstudy postprandial challenges with the test fat. In the latter situation, subjects were fed the test fats the in question for 4 weeks, either as an IE fat containing 18:0, or a standard saturated fat (eg, palm olein), or a partially hydrogenated fat with *trans* fatty acids. After 2 weeks into each fat period, subjects were fed a meal containing the study fat for an 8h postprandial evaluation. Thus, each individual rotated through all three test diets over a 12 week period. Each were their own controls, greatly increasing the potential to demonstrate a fat difference, and because the subjects had 2 week conditioning with the test fat prior to the meal and postprandial experience, the design was much more rigorous and apt to detect a response, especially since the IE fat was fed at relatively high intakes throughout the study, and was the only fat in the meal setting.

The IE sn2-18:0 fat in the Sundram report appeared to decrease insulin secretion and increase plasma glucose postprandially. In addition, a rise in *fasting* glucose was also encountered after 4 weeks, suggesting that reduced insulin availability may have caused glucose to rise. However, it could be that slower IE fat absorption postprandially lowered incretin production, while leaving CHO as the primary energy absorbed following the meal challenge, resulting in the observed glycemia. Since insulin resistance is thought to be the earliest evidence of T2DM, it likely would be the putative defect associated with the IE fat after 4 weeks. Insufficient data was collected to resolve these possibilities, and future research would do well to explore this avenue of inquiry. In

general, it is thought that consumption of extremely saturated fat, meaning minimal polyunsaturated fatty acid intake, has been linked to hyperglycemia and insulin resistance, but this too is somewhat tenuous (Riserus et al., 2009; Riccardi, 2004).

Furthermore since the Malay population studied is known to be prone to T2DM, the Sundram data might suggest that Malays represent an ideal phenotype for exploring metabolism of a poorly metabolized IE fat. For example, Lopez et al., 2011 and Christiansen et al., 1997 found major differences in insulin secretion based on the type of diet fat fed in hyperlipemic or diabetic subjects. Also, Pedersen et al. 1999 demonstrated that adding glucose to the fat in a test meal essentially acts like an oral glucose tolerance test in the presence of the test fat, indicating that both the amount and quality of the carbohydrate can affect postprandial glucose and insulin metabolism as well postprandial lipemia.

Fat and diabetes risk. The greatest association between dietary fat fatty acids and T2DM is definitely observed with *trans* fat consumption (Riserus et al., 2009). In addition, an Australian study in women (Hodge et al., 2007) assessed serum phospholipid fatty acids and dietary fat in relationship to T2DM. That study demonstrated a four-fold increased risk for diabetes in women having the highest 18:0 in their serum phospholipids, whereas the highest quintile for PL 18:2 reduced the risk to approximately 1/5 the amount of the lowest quintile of 18:2. These observations clearly suggest that fatty acids incorporated in phospholipids presumably can exert a positive or negative impact on diabetes risk, which ultimately should be reflected in the blood glucose and insulin levels. IE fats with sn2-SFA, especially since the sn2 FA gives rise to PL downstream, are highly suspect for T2DM risk until proven otherwise.

References

1. Abdul-Aziz K, Muniandy S, Shah I . Evaluation of Nε-(carboxymethyl)lysine and lipid peroxidation in multiethnic Malaysian subjects with type 2 diabetes mellitus Scientific Research and Essays 6: 1957-62, 4 May, 2011
2. Borkman M, Storlien LH, Pan DA, Jenkins AB, Chisholm DJ, Campbell LV. The relation between insulin sensitivity and the fatty-acid composition of skeletal-muscle phospholipids. N Engl J Med. 1993 , 328: 238-44.
3. Christiansen E, Schnider S, Palmvig B, Tauber-Lassen E, Pedersen O. Intake of a diet high in trans monounsaturated fatty acids or saturated fatty acids. Effects on postprandial insulinemia and glycemia in obese patients with NIDDM. Diabetes Care. 1997 20:881-7.
4. Hayes KC. Synthetic and modified glycerides: effects on plasma lipids. Curr Opin Lipidol. 2001 Feb;12(1):55-60. Review.
5. Hayes KC, Pronczuk A, Replacing trans fat: the argument for palm oil with a cautionary note on interesterification. J Am Coll Nutr 29, 253S–284S (2010)
6. Hodge AM, English DR, O'Dea K, Sinclair AJ, Makrides M, Gibson RA, Giles GG. Plasma phospholipid and dietary fatty acids as predictors of type 2 diabetes: interpreting the role of linoleic acid. Am J Clin Nutr. 2007 Jul;86(1):189-97.
7. Lee B, Gai W, Laychock S Proteasomal Activation Mediates Down-Regulation of Inositol 1,4,5-Trisphosphate Receptor and Calcium Mobilization in Rat Pancreatic Islets* Endocrinology 142: 1744–1751, 2001
8. Lovejoy JC, Smith SR, Champagne CM, Most MM, Lefevre M, DeLany JP, Denkins YM, Rood JC, Veldhuis J, Bray GA. Effects of diets enriched in saturated (palmitic), monounsaturated (oleic), or trans (elaidic) fatty acids on insulin sensitivity and substrate oxidation in healthy adults. Diabetes Care. 2002 Aug;25(8):1283-8.
9. Mensink RP, Katan MB. Trans monounsaturated fatty acids in nutrition and their impact on serum lipoprotein levels in man. Prog Lipid Res. 1993;32:111-22
10. Nilsson M, Stenberg M, Frid AH, Holst JJ, Björck IM. Glycemia and insulinemia in healthy subjects after lactose-equivalent meals of milk and other food proteins: the role of plasma amino acids and incretins. Am J Clin Nutr. 2004 Nov;80(5):1246-53
11. Nuttall FQ, Gannon MC. Effect of a LoBAG30 diet on protein metabolism in men with type 2 diabetes. Nutr Metab (Lond). 2012 May 20;9(1):43.

12. Pavkov ME, Hanson RL, Knowler WC, Bennett PH, Krakoff J, Nelson RG. Changing patterns of type 2 diabetes incidence among Pima Indians. Diabetes Care. 2007 Jul;30(7):1758-63.
13. Pedersen A, Marckmann P, Sandström B. Postprandial lipoprotein, glucose and insulin responses after two consecutive meals containing rapeseed oil, sunflower oil or palm oil with or without glucose at the first meal. Br J Nutr. 1999 Aug;82(2):97-104
14. Riccardi G, Giacco R, Rivellese AA. Dietary fat, insulin sensitivity and the metabolic syndrome. Clin Nutr. 2004 23:447-56. Review. Diabetes Metab. 1997 23: Suppl 4:6-9.
15. Risérus U, Willett W , Hu F . Dietary fats and prevention of type 2 diabetes. Progress in Lipid Research 48 (2009) 44–51.
16. Sundram K, Karupaiah T, Hayes KC. Stearic acid-rich interesterified fat and trans-rich fat raise the LDL/HDL ratio and plasma glucose relative to palm olein in humans. Nutr Metab (Lond). 2007 Jan 15;4:3.
17. Thomsen C, Storm H, Holst J, and Hermansen K. Differential effects of saturated and monounsaturated fats on postprandial lipemia and glucagon-like peptide 1 responses in patients with type 2 diabetes Am J Clin Nutr 2003;77:605–11.
18. Yli-Jokipii Y, Kallio H, Schwab U, Mykkänen H, Kurvinen J-P, Savolainen M, Tahvonen R Effects of palm oil and transesterified palm oil on chylomicron and VLDL triacylglycerol structures and postprandial lipid response J Lipid Res 2001. 42:1618–1625.
19. Zock PL , Katan MB. Hydrogenation alternatives: effects of trans fatty acids and stearic acid versus linoleic acid on serum lipids and lipoproteins in humans. J Lipid Res 1992. 33: 399-410.

ANTICIPATED PARTICIPANTS

1. Casimir Akoh	University of Georgia
2. David Baer	US Department of Agriculture
3. Douglas Balentine	Unilever
4. Ann Bouchoux	International Food Information Council
5. Robert Collette	Institute of Shortening & Edible Oils
6. Stephen Crozier	The Hershey Company
7. Richard D'Aloisio	Kraft Foods Inc.
8. Vincent de Jesus	US Food and Drug Administration
9. Brent Flickinger	Archer Daniels Midland Company
10. Courtney Gaine	ILSI North America
11. Suzanne Harris	ILSI Research Foundation
12. K.C. Hayes	Brandeis University
13. Eric Hentges	ILSI North America
14. Philip Howles	University of Cincinnati
15. Deb Kalisher	Mars Chocolate North America
16. Courtney Kelly	ILSI North America
17. Chor San Khoo	ILSI North America
18. David Klurfeld	USDA Agricultural Research Service
19. Elaine Krul	Solae LLC
20. Alejandro Marangoni	University of Guelph
21. Carey Mathesius	Pioneer Hi-Bred International Inc.
22. Ronald Mensink	Maastricht University
23. Linda Meyers	Institute of Medicine
24. Guillermo Napolitano	Nestlé PTC
25. Eric Park	Monsanto Company
26. Amy Preston	The Hershey Company
27. Kristin Rubin	Kraft Foods Inc.
28. Thomas Sanders	King's College London
29. Kris Sollid	International Food Information Council
30. Alison Steiber	Academy of Nutrition and Dietetics
31. Xuejun Tang	DSM Nutritional Products
32. Tom Tiffany	Archer Daniels Midland Company
33. Wei Wang-Nolan	DSM Nutritional Products
34. Robert Ward	Utah State University
35. Sarah Waybright	ILSI North America
36. Densie Webb	Freelance Writer
37. Peter Zock	Unilever Research & Development, Netherlands

****There will be additional participants via webinar****