Food & Function

Monoacylglycerol gel offers improved lipid profiles in high and low moisture baked products but does not influence postprandial lipid and glucose responses

Journal:	*Food & Function*
Manuscript ID:	FO-ART-11-2013-060596.R1
Article Type:	Paper
Date Submitted by the Author:	15-Jan-2014

Complete List of Authors:	Wright, Amanda J.; University of Guelph, Human Health & Nutrition Sciences Pinto, Christine; University of Guelph, Human Health & Nutritional Sciences Tulk, Hilary; University of Guelph, Human Health & Nutritional Sciences McCluskey, Julie; University of Guelph, Human Health & Nutritional Sciences Goldstein, Avi; University of Guelph, Department of Food Science Huschka, Brittany; University of Guelph, Human Health & Nutritional Sciences Marangoni, Alejandro; University of Guelph, Department of Food Science Seetharaman, Koushsik; University of Guelph, Department of Food Science

Title: Monoacylglycerol gel offers improved lipid profiles in high and low moisture baked products but does not influence postprandial lipid and glucose responses

Authors: Amanda Wright[1*], Christine Pinto[1], Hilary Tulk[1], Julie McCluskey[1], Avi Goldstein[2], Brittany Huschka[2], Alejandro Marangoni[2], and Koushik Seetharaman[2]

[1]Department of Human Health & Nutritional Sciences, University of Guelph, Guelph, ON, N1G 2W1

[2]Department of Food Science, University of Guelph, Guelph, ON, N1G 2W1

[*]Corresponding Author; ajwright@uoguelph.ca, 519-824-4120 x54697 (phone), 519-763-5902 (fax)

Abstract

Structured emulsions, including monoacylglycerol (MAG) gels, are of interest as alternatives to shortenings rich in saturated and *trans* fatty acids (SFA and TFA). However, an understanding of their physical and nutritional functionality in baked products is limited. The objective of this randomized crossover study was to compare the postprandial lipid and glucose responses to two different baked product matrices produced with a MAG gel. Differences between study treatments are discussed in the context of underlying ingredient interactions impacting, primarily, starch digestibility. Healthy males (n=18, 19-40 y, BMI ≤ 27 kg/m^2, waist circumference ≤ 102 cm, fasting plasma glucose < 5.6 mmoL/L, insulin < 180 pmol/L and TAG < 1.7 mmol/L) attended six study visits, each separated by at least one week, and consumed one of six study treatments with subsequent blood sampling for 6 h for determination of triacylglycerol (TAG), glucose, insulin and free fatty acids (FFA). The study treatments

consisted of sugar-free cakes and cookies (high and low moisture products, respectively) produced using either the canola oil-based structured MAG gel or the compositionally-equivalent MAG gel ingredients. Although MAG gel structure *per se* did not impact postprandial response, all cookies had higher TAG responses compared with cakes, even when matched for fat content. Sugar cookies containing 40 g of the MAG gel or an industry standard stearic-rich shortening were also compared, with no differences observed in postprandial response.

Introduction

Fat plays important functional roles in achieving the attributes that consumers expect from baked products. Saturated and *trans* fatty acids (SFA & TFA), specifically, provide desirable functionalities, including high melting points, increased lubricity, moisture barrier properties, creaming ability and storage stability[1-3] However, SFA and TFA consumption are associated with CVD,[4-6] insulin resistance[7, 8] and T2D.[7, 9] Therefore, food processors are motivated to offer products that are lower in SFA and TFA[10] but which do not compromise on the taste or quality achieved using traditional shortenings (e.g. hydrogenated fats, butter, margarine, lard). Structured emulsions, including monoacylglycerol (MAG) gels, are one strategy being pursued to allow the replacement of traditional shortenings with unsaturated liquid oils.[3, 11] It is now recognized that lipid structuring in foods, such as through emulsification, can impact bioavailability and metabolic response.[12]

We have previously reported on the production and properties of a semi-solid 60% oil MAG gel consisting of oil droplets surrounded by multilamellar monostearin water-entrapping layers. [3] When healthy human participants consumed the MAG gel (prepared with canola oil) versus compositionally equivalent oil-water mixtures (in the absence of MAG), attenuated postprandial

insulin, TAG and FFA responses were observed for.[3] In a subsequent study, participants consumed the MAG gel and compositionally equivalent oil-water mixtures on cooled toasted bread or warm pasta.[13] Attenuated postprandial TAG, FFA and insulin responses were observed when the MAG gel structure was consumed intact on the toast, but not with the pasta meal where the gel structure was lost due to heating.[13] Therefore, MAG gel structure may impact postprandial metabolism, although further research is required in order to discern compositional versus structural effects. The use of structured emulsions in foods may also impact metabolism indirectly by altering ingredient interactions during food processing. Specifically, the baking functionality of a MAG gel differs from traditional shortenings.[14, 15] Both the presence of liquid oil versus solid fat and a higher moisture content can affect ingredient interactions and impact gluten network formation. For example, traditional shortenings entrap and retain air during creaming, while liquid oils are dispersed throughout dough as globules, reducing the shortening and aeration effects.[14] Lipids also serve different purposes in different types of baked products. For example, in lower moisture products such as cookies, fat acts as a lubricant and competes with water to coat the surface of flour, preventing gluten hydration and network formation.[14] Shortening enables the incorporation of air into a batter during creaming and disrupts the continuity of starch and protein particles, thereby influencing cake tenderness and moisture.[16] In one study, replacing palm oil with MAG organogel and hydrogel yielded comparable or decreased quality characteristics, depending on the structuring strategy.[17] Therefore, the influence of structured emulsions may differ, depending on baked product matrix.

Sugar and starch also contribute to the desirable attributes of baked products. Sugar provides helps to incorporate air into fat during creaming, contributes to dough spread during baking and to the development of surface colour and texture, maintains moisture in prepared products and

provides sweetness.[18] However, overconsumption of sugar has been associated with elevated blood glucose levels, which contribute to CVD and T2D risk.[19, 20] The glycemic load of a baked product can be decreased by removing sugar or starch or replacing these ingredients with higher fibre carbohydrate sources. Unfortunately, this often has deleterious effects on product quality. Starch digestibility of baked products can vary for a number of reasons, including formulation differences, differences in kinetics of moisture removal during baking and microstructural differences.[21] For example, products made from higher moisture content doughs, such as cakes, experience more starch gelatinization during baking, than lower moisture content doughs like cookies.[22] Higher starch gelatinization has been associated with increased postprandial blood glucose and insulin.[23] The formation of amylose-lipid complexes during processing can also influence starch digestibility.[24-26] Therefore, the impact of structured emulsions such as MAG gels on starch digestibility and postprandial metabolism may differ between high and low moisture products.

This study with healthy adult men investigated the postprandial TAG, FFA, glucose and insulin responses to cookies and cakes prepared with the above-mentioned canola oil-based MAG gel. Consideration of postprandial metabolism is particularly important in terms of disease risk, given that we spend the majority of our time in a postprandial state[31] and that impaired postprandial regulation of blood lipids and glucose is associated with negative health outcomes.[4, 27-30] Sugar-free cookies and cakes, as examples of low and high moisture baked products, respectively, were prepared with either the structured MAG gel or the unstructured MAG gel ingredients to test the hypothesis that MAG gel structure (as opposed to composition), differentially based on product matrix, influences ingredient interactions during production and digestion that impact postprandial metabolism. To enable commercially relevant comparisons, sugar cookies, matched

for total amount of shortening, were also prepared with the structured MAG gel or with an industry standard high SFA shortening.

Experimental Study treatments

Table 1 shows the dough formulations for the 6 study treatments (4 types of cookies and 2 types of cake). These were developed specifically for the study and manufactured in the Bake Laboratory at the University of Guelph (Guelph, ON, CAN). The MAG gel was manufactured, as previously described[3] and stored at 20 °C for use within 7 days of production. It was based on canola oil (No Name®, Loblaws Company Ltd., Brampton, ON, CAN), stearic acid (Caravan Ingredients, Lenexa, KS, USA), deionized water and 90% distilled MAG (Caravan Ingredients, Lenexa, KS, USA). The MAG gel contained 7.35 % SFA, 33.80 % MUFA, 16.89 % PUFA, 5.27 % omega 3, 11.61 % omega 6, <0.5% TFA and 40 % water. An interesterified soy shortening (IE soy shortening, Archer Daniels Midland Company, Decutur, IL, USA) containing 45 % SFA, 16 % cis-MUFA, 39 % cis-PUFA and 1.5 % TFA (according to the manufacturer's specifications) was used as an industry standard shortening for comparison. All study treatments were prepared using the following ingredients; soft wheat flour (12 % moisture, 8 % protein, Griffith Laboratories, Toronto, ON, CAN), sucrose (Redpath Sugar Ltd., Toronto, ON, CAN), sodium bicarbonate (Arm & Hammer® Baking Soda, Church & Dwight Co., Inc., Princeton, NJ, USA), canola oil (No Name®, Loblaws Company Ltd., Brampton, ON, CAN), sodium chloride (Windsor®, The Canadian Salt Company Ltd., Pointe Claire, QC, CAN), and baking powder (Kraft Foods Inc., Northfield, IL, USA).

To test the hypothesis that the structure of the MAG gel, as opposed to its composition, impacts postprandial metabolism, sugar-free cookies and cakes were prepared by adding either the

structured MAG gel or the identical MAG gel ingredients (i.e. not formulated into a gel prior to mixing with other ingredients). The sugar-free cookies and cakes were prepared using a countertop Hobart mixer (Hobart Legacy HL120, Hobart Corporation, Troy, OH, USA), as follows. First, either the structured MAG gel or its unstructured ingredients and all dry ingredients were added to a Hobart mixing bowl. The ingredients were mixed for 2 min at speed #1, with bowl scraping every 30 s. Water was then slowly added while mixing at speed #1, followed by bowl scraping. For the cookies, the dough was mixed for 1 min at speed #2, with bowl scraping every 30 s. The dough was rolled using dough guides and cut into 50 g portions with a circular cookie cutter and baked at 204˚C for 24 min. For the cakes, the batter was mixed for 1.5 min at speed #2, with bowl scraping every 30 s. The batter was divided into 50 g portions and carefully baked at 185˚C for 45 min.

The sugar cookies were prepared according to a commercial recipe using the same mixer as above and as follows. Sucrose and the IE soy shortening were added to a Hobart mixing bowl and creamed for 30 s on speed #1, followed by bowl scraping. Baking soda and baking powder were added and the ingredients were mixed at speed #3 for 1 min, with bowl scraping every 30 s. Flour was added and the dough was mixed for 45 s on speed #2. The dough was rolled using dough guides and cut into 50 g portions with a circular cookie cutter. For the sugar cookies with the MAG gel, the structured MAG gel and all dry ingredients were added to a Hobart mixing bowl and mixed for 2 min at speed #1, with bowl scraping every 30 s. Water was then slowly added while mixing at speed #1. Mixing was continued for another 1 min at speed #2, with bowl scraping every 30 s. The dough was rolled using dough guides and cut into 50 g portions. Both types of sugar cookies were baked at 154˚C for 15 min. All study treatments were packaged in individual portions and stored in sealed plastic bags at -20 °C for up to 3 months prior to thawing

at room temperature the night before consumption. Fresh and frozen samples were analyzed for starch digestibility, as below, to verify no significant changes occurred in starch digestibility during the frozen storage ($P>0.05$, data not shown).

Table 2 shows the weights, moisture, fat and available carbohydrate compositions of the study treatments (i.e. four small cakes or cookies), as consumed by the study participants. The sugar-free cookies and cakes were matched for 40 g of fat and, respectively, contained ~ 90 versus 42 g available carbohydrate. The sugar cookies were matched for total shortening (42 g) and contained ~ 120 g available carbohydrates. Of note, because the MAG gel contains 60 % canola oil, the fat content of the sugar cookie with the MAG gel was 28 g.

The frozen products (once thawed) were analyzed for available carbohydrate, total starch, rapidly digestible starch (RDS), slowly digestible starch (SDS) and residual starch (ResS). Total starch was determined using the Megazyme Total Starch kit (Megazyme International Ireland, Bray, Ireland). For sugar-free cakes and cookies, the total starch value was used to calculate the available carbohydrate content. For sugar cookies, a combination of the total starch value and known sucrose addition levels were used to determine the available carbohydrate content *In-vitro* starch digestion fractions (RDS, SDS, ResS) were determined according to protocols described by Englyst et al. (1996)[32] with modifications. An enzyme mixture consisting of pancreatin from porcine pancreas (P-1625) (Sigma-Aldrich, Missouri, USA), invertase from baker's yeast (S. *sereviciae*) (Sigma-Aldrich, Missouri, USA) and amyloglucosidase (200 U/mL) (Megazyme International Ireland, Bray, Ireland) was prepared according to Englyst et al. (1992).[32] 0.5 g of thawed sample were added to a round bottom flask along with 10 mL 0.1M sodium acetate buffer (pH 5.2). After a brief incubation at 37 °C, 5 mL enzyme mixture was added to round bottom flasks. The sample and enzyme mixture was incubated for 2 h with 0.1

mL aliquots taken every 20 min. The aliquots were transferred into 80% ethanol to stop the hydrolysis, and stored in a -20 °C freezer. The amount of glucose released was determined by glucose oxidase/peroxidase analysis. Hydrolyzed carbohydrates were classified into RDS (glucose released at 20 min), SDS (glucose released at 120 min) and ResS (total Glucose - (RDS + SDS)). The concepts of RDS, SDS and ResS relate to the underlying microstructure of starch and can be used to characterize the susceptibility of starch to digestion in the small intestine.[26] Significant differences in RDS and SDS were observed ($P<0.05$, Table 2). Specifically, SDS was lower in the cakes versus cookies ($P<0.05$). Moisture was determined using the AG-AACC Method 44-40.01.[33] Reported fat contents are based on calculation and confirmed via Soxhlet extraction with petroleum ether.

Thawed samples of each study treatment were ground and sieved through a 850 μm mesh for analysis by x-ray diffraction. X-ray diffractrograms were obtained by radiation produced by a Cu ($K\alpha_1 = 0.154$ Å) X-ray tube within a Rigaku X-ray diffractometer (Rigaku-Denki, Co., Tokyo, Japan). The diffraction patterns were obtained with the following conditions: target voltage 40 kV, current 40 mA, scan speed 1˚/min, sampling width 0.02˚, divergence slit width 0.5nm, scatter slit width 0.5nm, receiving slit width 0.3 nm, and scanning range 3–35θ. Resulting diffraction patterns were smoothed using Jade Software (version 6.5, Material Data Inc., California, USA). Characteristic peaks for A-type crystals (2θ 15.1, 17.5, and 23) and V-type amylose-lipid complex (2θ 8.1, 12.8, 19.8) were obtained, in agreement with reports of Zobel et al.[34] Figure 1 shows representative x-ray diffraction patterns obtained for the sugar-free cookies and cakes produced with the MAG gel.

Human study design

The study utilized a randomized crossover design and was double-blinded within the cookie and the cake treatments. Participants consumed 1 of 6 study treatments at each of the 6 trial days, which were separated by a period of at least 5 days. This study was approved by the University of Guelph Human Research Ethics Board and all participants provided written informed consent.

Study participants

Healthy male participants (19-40 y, BMI ≤ 27 kg/m^2, waist circumference ≤ 102 cm, fasting plasma glucose < 5.6 mmoL/L, insulin < 180 pmol/L and TAG < 1.7 mmol/L) were recruited from the Guelph community. Exclusion criteria included smoking, the use of medications, natural health products or recreational drugs as well as irregular eating habits (assessed by 3-day food records). Vegetarians, vegans, men with food allergies or intolerances or diagnosed medical conditions, as well as elite athletes and men training for an athletic event, were excluded from participation. In total, 19 participants enrolled in the study, with 17 participants completing all 6 treatments and 2 participants withdrawing after two treatments due to scheduling conflicts.

The clinical trial

Participants were asked to maintain their usual lifestyle and eating habits for the duration of the study and were instructed to specifically avoid alcohol, caffeine and strenuous physical activity for 48 h prior to each study visit. The evening prior to each study visit, participants were provided with a standardized meal to consume between 6:00 and 8:00 pm (593.0 kcal, 5.4 % protein, 15.8 % carbohydrate, 2.7 % fat) and then observed a 12-14 h overnight fast. Immediately prior to each study visit, participants completed 3-day food records, which were analyzed to determine 3-day average intakes of energy, energy from fat, total fat, SFA, TFA, MUFA, PUFA, cholesterol, protein, carbohydrate and dietary fibre (ESHA Food Processor SQL v10.5, ESHA

Research, OR, USA, P>0.05, data not shown).

For each study visit, participants reported to the clinical trial suite (Human Nutraceutical Research Unit, University of Guelph, Guelph, ON, CAN). Fasting body weight was determined to the nearest 0.1 kg at each study visit using a digital scale (SVI 200F; Acculab®, Bohemia, NY, USA). Height, waist and hip circumference, blood pressure, and body composition were determined at study visits one and six. Waist circumference was measured at the midpoint between the lowest palpable rib and the iliac crest and hip circumference was measured at the level of the greater trochanter both to the nearest 0.1 cm using an inelastic measuring tape (Seca 201, SECA, Hamburg, Germany). Blood pressure was measured in duplicate (OMRON Intellisense® Professional Digital Blood Pressure Monitor HEM-907XL, Bannockburn, IL, USA) and the results averaged. Body composition (% body fat) was determined by bioelectrical impedance (BodyStat®1500; BodyStat Ltd., Douglas, IOM).

To facilitate blood collection, a closed intra-venous catheter system (BD Saf-T-Intima™, Becton Dickinson, Franklin Lakes, NJ, USA) was inserted in the antecubital region of each participant's arm. Following a fasting (0 min) blood sample, participants were given 10 min to consume one study treatment with 250 mL of room temperature water. Subsequent blood samples were procured at 30, 60, 90, 120, 150, 180, 240, 300 and 360 min. Participants remained seated during the study visit with only short walks to use the washroom permitted, as necessary.

For glucose analysis, blood was collected in 2 mL sodium heparin tubes (BD Vacutainer™, Becton Dickinson, Franklin Lakes, NJ, USA), placed on ice and then centrifuged (2400 rpm, 10 min, 4 °C) within 30 min. Plasma glucose was analyzed in duplicate using the glucose oxidase method (YSI 2300 STAT Plus^{TM}, YSI Inc. Life Sciences, Yellow Springs, OH, USA).

For determination of serum lipids, blood was collected in 5 mL standard serum tubes (BD Vacutainer™, Becton Dickinson, Franklin Lakes, NJ, USA), allowed to clot at room temperature for 30 min and then centrifuged (3000 rpm, 15 min, 4 °C). Serum was held at 4 °C for same day pick-up and analysis at an external laboratory (LifeLabs® Medical Laboratory Services, Toronto, ON, CAN) using a Siemens ADVIA® Chemistry Systems and reagents (Siemens Healthcare Diagnostics, Deerfield, IL, USA). For each trial day, fasting TAG, HDL- and total cholesterol were determined, and LDL-cholesterol was calculated.[35] Maximum intra-assay variation was 3% for total cholesterol, HDL-cholesterol and TAG.

For insulin and FFA analysis, blood was collected in 5 mL no-additive serum tubes (BD Vacutainer™, Becton Dickinson, Franklin Lakes, NJ, USA), allowed to clot at room temperature for 30 min, and centrifuged (3000 rpm, 15 min, 4 °C). Two 1 mL aliquots of serum were partitioned and frozen at -80 °C for later analysis. Serum insulin was measured in duplicate using a radioimmunoassay (Coat-A-Count®, Siemens Medical Solutions Diagnostics, Los Angeles, CA, USA) with inter- and intra-assay variations of 4.9 - 10 % and 5 - 9.3 %, respectively. Serum FFA was measured in duplicate using an *in vitro* enzymatic colourimetric method assay (Wako HR Series NEFA-HR, Richmond, VA, USA) with inter- and intra-assay variations of 0.36 ± 0.03 mEq/L and 0.34 ± 0.16 mEq/L, respectively.

Data and statistical analysis

Data for one participant was excluded from the analysis because fasting TAG exceeded the 1.7 mmol/L cut off on 5 of the 6 study visit days. Two participants withdrew from the study due to scheduling conflicts, each having completed 2 study visits. Data from their completed study days

was included in the analysis. Therefore data from 18 participants was included in the statistical analysis.

Participant characteristics (Table 3), including height, body weight, BMI, waist circumference, blood pressure and body fat, as well as fasting glucose and insulin concentrations were compared between study visits 1 and 6 using paired t-tests ($P>0.05$). 3-day food records were analyzed using ESHA Food Processor SQL v9.04 software and means of energy, energy from fat, total fat, SFA, TFA, MUFA, PUFA, cholesterol, protein, carbohydrate and dietary fibre were compared between each study visit using a one-way repeated measures ANOVA (Prism® v5.0, GraphPad Software, La Jola, CA).

Fasting and postprandial glucose, insulin, TAG and FFA concentrations were plotted as a function of time and incremental area-under-the-curve (AUC), time-to-peak and peak values were determined. AUC considered all area above baseline over 360 min for glucose, insulin and TAG and all area below baseline over 360 min for FFA. AUC was calculated using the trapezoidal method (Prism® v5.0, GraphPad Software, La Jola, CA). Time-to-peak was defined as the time at which the highest value for an endpoint occurred and was determined by visual inspection.

Unless otherwise stated, all statistical analyses were performed using Statistical Analysis System version 9.1 (SAS Institute, Inc., Cary, NC) and using a significance level of $P \leq 0.05$. The summary statistics (AUC, time-to-peak and peak value) for glucose, insulin, TAG and FFA were examined for normality using box plots, stem leaf diagrams and residual error plots. All data were transformed using the natural logarithm prior to statistical analysis to better comply with the normality and equal variance assumptions underlying the statistical analysis. For

presentation, the anti-log of the transformed mean (geometric mean) was calculated, along with the SEM appropriate on the log scale.

The effect of treatment for AUC, time-to-peak and peak value was statistically analyzed as a crossover design, blocking for participant and trial day. There was no effect of trial day ($P>0.05$). Baseline value was found to have a significant effect on TAG and insulin peak values only and was therefore included as a covariate in these analyses. An initial F-test was used to determine the effect of treatment for AUC, time-to-peak and peak value. Contrasts included the main effects of product moisture (sugar-free cakes versus all cookies) and of MAG gel structure (within the sugar-free cakes and sugar-free cookies), as well as interactions between product moisture and MAG gel structure. Contrasts were also used to compare the sugar-free versus sugar cookies.

Results

Table 4 summarizes the contrast testing results for the study main effects and Figures 2, 3, 4 and 5 show the postprandial TAG, glucose, insulin and FFA results, respectively. There were no differences in any postprandial parameters between the sugar-free cakes and cookies prepared with the structured MAG gel (SF-Cake-MAG & SF-Cookie-MAG) versus the unstructured compositionally-equivalent MAG gel ingredients ($P>0.05$, Table 4, data not shown). Therefore, only the data for the SF-Cake-MAG & SF-Cookie-MAG are shown in Figures 2-5.

There was no difference in TAG response between any of the cookies ($P>0.05$). TAG AUC and peak values were higher for the sugar-free cookies versus the sugar-free cakes ($P<0.05$, Figure 2B and 2C), despite being matched for total fat content. There were no differences in glucose AUC or time to peak values between any of the cookies ($P>0.05$). Glucose AUC was higher for

the sugar-free cookies versus sugar-free cakes ($P<0.05$, Figure 3) and there was a faster return to baseline for the sugar-free cakes (101.3 ± 38.7 min) versus sugar-free cookies (126.6 ± 59.2 min) ($P<0.05$, data not shown). No differences in postprandial glucose or insulin (Figures 3 and 4) were observed between the sugar cookies prepared with the MAG gel and IE soy shortening ($P>0.05$). Insulin AUC, peak and time to peak values were lower for the sugar-free cakes compared to the sugar-free cookies ($P<0.05$, Figure 4). The sugar-free cookie had a lower peak value, but higher time to peak value relative to the sugar cookie also prepared with the MAG gel ($P<0.05$). There were no differences in postprandial FFA between any of the treatments ($P>0.05$). While FFA appeared to return to baseline faster following consumption of the cakes, there were no significant differences in terms of time for return to baseline ($P=0.19$) or FFA value at 360 min ($P=0.08$).

Discussion

This study is the first to examine postprandial CVD and T2D biomarkers following consumption of high and low moisture baked products (i.e. cakes and cookies, respectively) containing a structured emulsion. It contributes to an understanding of how lipid structuring and food matrix impact lipid and carbohydrate absorption.

Effect of MAG gel structure

Consumption of the MAG gel in the form of a spread was previously shown to attenuate postprandial TAG, FFA and insulin responses, in comparison to compositionally-equivalent mixtures of oil and water.[3] In the present study, there was no effect of MAG gel structure on postprandial metabolism ($P>0.05$), within either the sugar-free cakes or cookies. Similarly, when the structured emulsion was melted prior to being consumed as part of a mixed meal, no

differences in postprandial TAG, FFA or insulin were observed.[13] Therefore, the presence of an intact structure plays a role in modulating postprandial biomarkers of chronic disease.

Loss of the MAG gel structure during baking was not unexpected. Structural changes could occur with mixing[36] interactions with flour components such as gluten[15] and heat. However, it was hypothesized that use of the structured MAG gel versus the MAG gel ingredients in an unstructured form would lead to different interactions during the mixing and baking processes, with potential implications for digestibility and, in turn, postprandial metabolism. According to Table 2, there were differences in RDS between the sugar-free cookies produced with the compositionally-equivalent MAG gel ingredients (57.5 %) versus the pre-formed MAG gel (52.5 %, $P<0.05$). Figure 1 also shows that remnants of the A-type crystalline pattern in starch were more prominent in the cookies baked with the structured MAG gel versus the MAG gel ingredients. Both results are consistent with the presence of free water in the non-gel scenario, as determined by analysis of their ^{1}H NMR T2 relaxation profiles[36] leading to more gelatinization. However, the same trend was not observed with the higher moisture products, i.e. the cakes. As expected, no remnants of native crystalline starch were observed in either cake tested, according to XRD. Substantially more Res-S was formed when the compositionally equivalent ingredients were used to produce the cakes instead of the preformed MAG gel. This could be due to more extensive gelatinization and therefore likely a higher degree of starch retrogradation.[37] Of note, despite the small differences in SDS and Res-S between the study treatments produced with the structured and unstructured MAG gel (Table 2), there were no differences in postprandial glucose or insulin responses within either product type ($P>0.05$, Table 4).

Comparison of sugar-free cakes versus cookies

As expected, glucose response was different between the sugar-free cakes (SF-Cake-MAG) and cookies (SF-Cookie-MAG), i.e. AUC was lower for the cakes. However, the differences were minimal given that the cookies contained more than twice the amount of available carbohydrates as the cakes (i.e. ~ 90 versus 41 g) and may be attributed to differences in the underlying product matrices and, in particular, the influence of water. Specifically, the cakes had a much higher moisture content. A higher availability of water leads to more starch granule swelling and gelatinisation, making the starch more susceptible to amylolytic enzymes during digestion.[23, 38-40] Starch in cake products tends to be more highly gelatinized than in cookies and other low moisture products.[26] One of the cakes (SF-Cake-MAG) had more RDS than the cookies, consistent with the observed earlier glucose time-to-peak for cakes. Similarly, the cakes had both significantly less SDS than the cookies ($P<0.05$) and a lower overall carbohydrate load which is consistent with the observed trend towards a more rapid return to baseline glucose for the cakes versus cookies (Figure 3A). A slower rate of glucose absorption is favourable due to decreased postprandial levels of induced gut hormones (i.e. incretins) and insulin. The insulin trends between the cakes and cookies were very similar to the glucose results. Figure 6 shows the positive and linear correlations observed between available carbohydrate content and glucose and insulin AUC, on both wet and dry weight bases. The FFA response for all study treatments was inversely related to the insulin response (Figures 3A and 4A),[41] with the cakes trending towards a more rapid return to baseline than the cookies (Figure 4A). This was expected, given that the cakes contained less carbohydrate than the cookies, which reduces the insulin demand.[42] In turn, a lower insulin response induces less suppression of FFA release.[43, 44]

The bell-shaped TAG response profiles observed for all study treatments (Figure 2A) are consistent with those observed in similar studies with healthy adults.[45, 46] TAG AUC and peak

values were higher for all the cookies compared with the cakes, despite being matched for 40 g canola oil (28 g in the case of S-Cookie MAG). This suggests that the cakes' higher moisture content contributed to a blunted TAG response. Postprandial TAG responses were the same following consumption of the sugar cookies containing approximately 40 g IE soy shortening (S-Cookie-IE, containing approximately 42 g stearic-rich fat) or MAG gel (S-Cookie-MAG, containing 27.5 g canola oil). Together, these results suggest that product moisture had a greater impact on the postprandial TAG response than either the amount or type of fat present in the sugar cookies.

The lower TAG response for the sugar-free cakes compared with the sugar-free cookies (matched for 40 g lipid) was somewhat surprising. On a dry weight basis, the cakes, as consumed, contained approximately 43 % lipid compared with the sugar free cookies which contained approximately 25% lipid. Figure 7 shows the non-linear relationship between TAG, glucose and insulin response as a function of available carbohydrate:fat ratio across all test meals, on both dry and wet weight bases. The differences in TAG response based on product matrix could, in part, be related to the higher available carbohydrates in the cookies. However, while d*e novo* lipogenesis can occur following consumption of carbohydrates,[47, 48] particularly fructose,[49] it would not account for the near doubling of TAG AUC observed between sugar free cakes and cookies, given that the study involved healthy individuals whom consumed moderate levels of carbohydrates which were derived from starch, in the case of the sugar-free cookies and cakes. Although, higher insulin AUC for the cookies may have contributed to the observed exacerbated postprandial TAG, Harbis et al. (2001)[50] noted that, in healthy individuals, physiological levels of insulinemia induced by starchy foods do not induce noticeable changes in TAG response.

Differences in gastrointestinal bolus volume are expected between some of the study treatments and, through impacting gut hormones and satiety signals, could contribute to differences in postprandial metabolism, as measured. The differences in postprandial TAG between the cakes and cookies could also be related to product matrix effects and factors such as spatial location of fat and protein within the food bolus,[26] physical barriers to enzyme access, and viscosity of gastrointestinal contents that might affect processes like mass transport or gastrointestinal emptying.[26] Molecular complexes between amylose and lipids can form during processing and/or during digestion and decrease starch digestibility.[26] According to XRD, more amylose-lipid complexes were present in the baked cakes versus cookies. This was expected based on the higher moisture content leading to more starch gelatinization[51] effectively enabling greater access for lipids to complex with amylose. It may also help to explain the relatively lower postprandial TAG for the cakes, assuming the formation of complexes effectively decreased the availability of lipids for digestion. However, as discussed above, there was no corresponding attenuation of postprandial glucose or insulin for the cakes with higher Res-S (S-Cake-MAG gel ingredients), as might have been expected if amylose molecules were less available for digestion.[52, 53] Since total plasma TAG analysis includes contributions from the intestinally-derived chylomicrons and liver-derived very low density lipoproteins, the differences observed following consumption of the products could be related to differences in the appearance and clearance of these different TAG-rich lipoproteins. The relationships between food microstructure, *in vitro* and *in vivo* nutrient digestibility and physiological response are complex. These results point to differing postprandial TAG, an important CVD biomarker, to the same load of dietary fat, depending on product moisture content and underlying structure.

Comparison of cookies

Glucose and insulin AUC values did not differ between any of the cookies, despite the large differences in available carbohydrate content (i.e. 41 - 124 g, Table 2). There were differences observed in peak glucose and insulin values between the MAG gel sugar and sugar-free cookies ($P<0.05$), which were consistent with the higher load of available carbohydrates. There were no differences observed in starch digestibility between the sugar cookies (Table 2). This suggests the MAG gel behaved similarly to the IE soy shortening during gelatinization and formation of the sugar cookie structure. Thus differences between the treatments in terms of starch digestibility, did not exist (Table 2). Importantly, healthy individuals, such as in this study, are generally able to metabolize mixed meals without large changes in postprandial response,[54] thereby making it difficult to differentiate treatment effects. There may be greater potential to observe metabolic differences in individuals who are predisposed to insulin resistance, have high adiposity or low physical activity levels.[55]

We considered that there could have been a hypertriglyceridemic effect for the sugar cookies, based on a contribution from fructose in the sucrose.[56-59] However, there were no differences in TAG or FFA response between any of the cookies. This was true, despite the fact that the sugar cookies also contained different fatty acid profiles. The MAG gel sugar cookies contained canola oil (i.e. approximately 7.4 % SFA and 33.5 % MUFA) and those produced with the commercial shortening contained approximately 45.0 % SFA and 16.0 % MUFA. Previous studies have shown similar, if not higher, postprandial TAG and FFA following consumption of MUFA- versus SFA-rich meals because of relatively lower SFA absorption.[60-62] No differences were observed, although the effects could have been mitigated by the fact that the S-Cookie-MAG contained 27.5 g highly monounsaturated oil whereas the S-Cookie-IE contained 42.1 g stearic acid-rich fat. There is evidence that the fatty acid composition of a mixed meal can impact the

glycemic and insulinemic responses to carbohydrate,[63] although there also were no differences in postprandial glucose or insulin responses between the sugar cookies in the present study. Still, confounding effects based on differing composition between products constitutes a potential limitation of the study.

Conclusion

Structured emulsions, including MAG gels, are of interest for baked products, given the potential to utilize unsaturated oils in place of traditional hardstocks and for lower fat contents. This research contributes to an understanding of postprandial metabolism following consumption of different baked product matrices in healthy males. Sugar-free cookies had higher glucose and insulin AUC values, but also much higher TAG AUC values compared to sugar-free cakes of similar fat, but much lower available carbohydrate contents. No differences were observed when the compositionally-equivalent MAG gel ingredients were used in place of the structured MAG gel to produce sugar-free cakes or cookies. Differences, however, may be observed with foods, such as spreads or condiments, where the structured emulsion is consumed intact. There were no differences in postprandial metabolism, as studied, when a MAG gel replaced a highly saturated industry standard shortening in a commercial recipe sugar cookie. However, longer term reductions in dietary fat consumption, specifically SFA and TFA, led to improvements in circulating lipids and CVD risk.[54, 64] Therefore, regular consumption of baked goods based on the MAG gel would have anticipated health benefits, while also enabling cleaner labels for food manufacturers.[1]

Acknowledgements

Thank you to our study participants for their time and dedication and to Mehrnoosh Kashani, Amiee Hill, Justine Tishinsky, Krista Dewsbury, Jennifer Biesel, Veronika DaSilva, Jennifer Laporte, Tanicha Langlois, Jessica Pask, Carolyn Daoust, Tara Galloro and Aleksandra Gasinski for their technical assistance. The statistical consulting services of Brian Allen and James Zhang of the Ashton Statistical Laboratory at the University of Guelph are gratefully acknowledged. Funding was provided by the Ontario Ministry of Agriculture, Food and Rural Affairs.

References

1. Eckel RH, Borra S, Lichtenstein AH, Yin-Piazza SY. Understanding the complexity of trans fatty acid reduction in the american diet: American heart association trans fat conference 2006: Report of the trans fat conference planning group. April 24, 2007; United States: ; 2007.

2. Ascherio A, Willet W. Health effects of*trans*fatty acids. The American Journal of Clinical Nutrition. 1997;66:1006S-1010S.

3. Marangoni AG, Idziak SHJ, Vega C, Batte H, Ollivon M, Jantzi PS, et al. Encapsulation-structuring of edible oil attenuates acute elevation of blood lipids and insulin in humans. Soft Matter. 2007;3:183-187.

4. Berry SE, Sanders TA. Influence of triacylglycerol structure of stearic acid-rich fats on postprandial lipaemia. Nutrition Society.Proceedings. 2005 May 2005;64(2):205-12.

5. Erkkila A, De Mello, V. D. F., Riserus U, Laaksonen DE. Dietary fatty acids and cardiovascular disease: An epidemiological approach. Progress in lipid research. 2008;47:172-187.

6. Hu FB, Willett WC. Optimal diets for prevention of coronary heart disease. JAMA. 2002 Nov 27, 2002;288(20):2569-78.

7. Due A, Larsen TM, Hermansen K, Stender S, Holst JJ, Toubro S, et al. Comparison of the effects on insulin resistance and glucose tolerance of 6-mo high-monounsaturated-fat, low-fat, and control diets. American journal of clinical nutrition AJN. 2008 April 2008;87(4):855-62.

8. Haag M, Dippenaar NG. Dietary fats, fatty acids and insulin resistance: Short review of a multifaceted connection. Medical science monitor : international medical journal of experimental and clinical research. 2005 December 2005;11(12):RA359-67.

9. Risérus U, Willett WC, Hu FB. Dietary fats and prevention of type 2 diabetes. Prog Lipid Res. 2009 January 2009;48(1):44-51.

10. Ramli MR, Lin SW, Yoo CK, Idris NA, Sahri MM. Physico-chemical properties and performance of high oleic and palm-based shortenings. Journal of oleo science. 2008;57(11):605-12.

11. Heertje I, Roijers EC, Hendrickx HACM. Liquid crystalline phases in the structuring of food products. Lebensmittel-Wissenschaft+Technologie.Food science+technology. 1998;31(4):387-96.

12. Michalski MC, Genot C, Gayet C, Lopez C, Fine F, Joffre F, et al. Multiscale structures of lipids in foods as parameters affecting fatty acid bioavailability and lipid metabolism. Prog Lipid Res. 2013 10;52(4):354-73.

13. Rush JWE, Jantzi PS, Dupak K, Idziak SHJ, Marangoni AG. Effect of food preparation on the structure and metabolic responses to a monostearin-oil-water gel-based spread. Food research international. 2008 December 2008;41(10):1065-71.

14. Goldstein A, Seetharaman K. Effect of a novel monoglyceride stabilized oil in water emulsion shortening on cookie properties. Food research international. 2011 Jun 2011;44(5):1476-81.

15. Huschka B, Challacombe C, Marangoni AG, Seetharaman K. Comparison of oil, shortening, and a structured shortening on wheat dough rheology and starch pasting properties. Cereal Chem. 2011 May/Jun 2011;88(3):253-9.

16. Painter KA. Functions and requirements of fats and emulsifiers in prepared cake mixes. Journal of the American Oil Chemists' Society. 1981 February 1981;58(2):92-5.

17. Calligaris S, Manzocco L, Valoppi F, Nicoli MC. Effect of palm oil replacement with monoglyceride organogel and hydrogel on sweet bread properties. Food Research International. 2013;51(2):596-602.

18. Pareyt B, Delcour JA. The role of wheat flour constituents, sugar, and fat in low moisture cereal based products: A review on sugar-snap cookies. Critical reviews in food science and nutrition. 2008 October 2008;48(9):824-39.

19. Meigs JB, Nathan DM, D'Agostino RB, Wilson PWF. Fasting and postchallenge glycemia and cardiovascular disease risk: The framingham offspring study. Diabetes Care. 2002 October 2002;25(10):1845-50.

20. Riccardi G, Rivellese AA, Giacco R. Role of glycemic index and glycemic load in the healthy state, in prediabetes, and in diabetes. American journal of clinical nutrition AJN. 2008 January 2008;87(1):269S-74.

21. Goldstein A, Nantanga KKM, Seetharaman K. Molecular interactions in starch-water systems: Effect of increasing starch concentration. Cereal Chem. 2010 Jul/Aug 2010;87(4):370-5.

22. Walker S, Seetharaman K, Goldstein A. Characterizing physiochemical changes of cookies baked in a commercial oven. Food Research International. 2012;48(1):249-56.

23. Holm J, Lundquist I, Björck I, Eliasson AC, Asp NG. Degree of starch gelatinization, digestion rate of starch in vitro, and metabolic response in rats. Am J Clin Nutr. 1988 June 1988;47(6):1010-6.

24. Bjorck I, Granfeldt Y, Liljeberg H, Tovar J, Asp NG. Food properties affecting the digestion and absorption of carbohydrates. American journal of clinical nutrition. 1994 March 1994;59(3S):699S-705.

25. Ai Y, Hasjim J, Jane J. Effects of lipids on enzymatic hydrolysis and physical properties of starch. Carbohydr Polym. 2013 January 30, 2013;92(1):120-7.

26. Parada J, Aguilera JM. Review: Starch matrices and the glycemic response. Food Sci Technol Int. 2011 Jun 2011;17(3):187-204.

27. Eberly LE, Stamler J, Neaton JD. Relation of triglyceride levels, fasting and nonfasting, to fatal and nonfatal coronary heart disease. Arch Intern Med. 2003 May 12, 2003;163(9):1077-83.

28. Kolovou GD, Mikhailidis DP, Kovar J, Lairon D, Nordestgaard BG, Ooi TC, et al. Assessment and clinical relevance of non-fasting and postprandial triglycerides: An expert panel statement. Current vascular pharmacology. 2011 May 2011;9(3):258-70.

29. Paglialunga S, Cianflone K. Regulation of postprandial lipemia: An update on current trends. Applied Physiology, Nutrition, and Metabolism. 2007 Feb 2007;32(1):61-75.

30. Sanders TAB. Dietary fat and postprandial lipids. Curr Atheroscler Rep. 2003 November 2003;5(6):445-51.

31. Lairon D. Macronutrient intake and modulation on chylomicron production and clearance. Atherosclerosis.Supplements. 2008 September 2008;9(2):45-8.

32. Englyst HN, Kingman SM, Cummings JH. Classification and measurement on nutritionally important starch fractions. European journal of clinical nutrition. 1992 October 1992;46:S33-50.

33. AACC I. International approved methods of analysis. method 44e40.01. 2010.

34. Zobel HF, Young SN, Rocca LA. Starch gelatinization: An X-ray diffraction study. Cereal chemistry. 1988 November 1988;65(6):443-6.

35. Friedewald WT, Levy RI, Fredrickson DS. Estimation of the concentration of low-density lipoprotein cholesterol in plasma, without use of the preparative ultracentrifuge. Clin Chem. 1972 June 1972;18(6):499-502.

36. Goldstein A, Marangoni A, Seetharaman K. Monoglyceride stabilized oil in water emulsions: An investigation of structuring and shear history on phase behaviour. Food Biophysics. 2012 Sep 2012;7(3):227-35.

37. Stute R:H, Klinger RW, Boguslawski S, Eshtiaghi MN, Knorr D. Effect of high pressure treatment on starches. Starch/Starke. 1996;48:399-408.

38. Bornet FRJ, Fontvieille AM, Rizkalla S, Colonna P, Blayo A, Mercier C, et al. Insulin and glycemic responses in healthy humans to native starches processed in different ways: Correlation with in vitro alpha-amylase hydrolysis. American journal of clinical nutrition. 1989 August 1989;50(2):315-23.

39. Garsetti M, Vinoy S, Lang V, Holt S, Loyer S, Brand-Miller JC. The glycemic and insulinemic index of plain sweet biscuits: Relationships to in vitro starch digestibility. Journal of the American College of Nutrition. 2005 December 2005;24(6):441-7.

40. Nieuwenhuijzen NHv, Meinders MBJ, Tromp RH, Hamer RJ, Vliet Tv. Water uptake mechanism in crispy bread crust. Journal of agricultural and food chemistry. 2008 August 13, 2008;56(15):6439-46.

41. Jelic K, Hallgreen CE, Colding-Joergensen M. A model of NEFA dynamics with focus on the postprandial state. Ann Biomed Eng. 2009 Sep 2009;37(9):1897-909.

42. Liljeberg HGM, Bjorck IME. Delayed gastric emptying rate as a potential mechanism for lowered glycemia after eating sourdough bread: Studies in humans and rats using test products with added organic acids or an organic salt. Am J Clin Nutr. 1996 Dec 1996;64(6):886-93.

43. Kiens B, Richter EA. Types of carbohydrate in an ordinary diet affect insulin action and muscle substrates in humans. Am J Clin Nutr. 1996 Jan 1996;63(1):47.

44. Jensen MD, Heiling V, Miles JM. Measurement of non-steady-state free fatty acid turnover. Am J Physiol. 1990 January 1990;258(1 Pt 1):E103-8.

45. Radulescu A, Hassan Y, Gannon MC, Nuttall FQ. The degree of saturation of fatty acids in dietary fats does not affect the metabolic response to ingested carbohydrate. Journal of the American College of Nutrition. 2009;28(3):286-295.

46. Rifai N, Merrill JR, Holly RG. Postprandial effect of a high fat meal on plasma lipid, lipoprotein cholesterol and apolipoprotein measurements. Ann Clin Biochem. 1990 September 1990;27 (Pt 5):489-93.

47. Lopez-Miranda J, Marin C. Dietary, physiological and genetic impacts on postprandial lipid metabolism. In: Montmayeur J, Le Coutre J, editors. Fat detection : taste, texture, and post ingestive effects. CRC Press/Taylor & Francis; 2010.

48. Parks EJ, Hellerstein MK. Carbohydrate-induced hypertriacylglycerolemia: Historical perspective and review of biological mechanisms. American journal of clinical nutrition. 2000 February 2000;71(2):412-33.

49. Chong MFF, Fielding BA, Frayn KN. Mechanisms for the acute effect of fructose on postprandial lipemia. American journal of clinical nutrition AJN. 2007 June 2007;85(6):1511-20.

50. Harbis A, Defoort C, Narbonne H, Juhel C, Senft M, Latgé C, et al. Acute hyperinsulinism modulates plasma apolipoprotein B-48 triglyceride-rich lipoproteins in healthy subjects during the postprandial period. Diabetes. 2001 February 2001;50(2):462-9.

51. Jovanovich G, Zamponi RA, Lupano CE, Anon MC. Effect of water content on the formation and dissociation of the amylose-lipid complex in wheat flour. Journal of agricultural and food chemistry. 1992 October 1992;40(10):1789-93.

52. Hasjim J, Lee S, Hendrich S, Setiawan S, Ai Y, Jane J. Characterization of a novel resistant-starch and its effects on postprandial plasma-glucose and insulin responses. Cereal chemistry. 2010 Jul 2010;87(4):257-62.

53. Takase S, Goda T, Watanabe M. Monostearoylglycerol-starch complex: Its digestibility and effects on glycemic and lipogenic responses. J Nutr Sci Vitaminol. 1994 February 1994;40(1):23-36.

54. Siri-Tarino PW, Sun Q, Hu FB, Krauss RM. Meta-analysis of prospective cohort studies evaluating the association of saturated fat with cardiovascular disease. American journal of clinical nutrition AJN. 2010 March 2010;91(3):535-46.

55. Mozaffarian D, Aro A, Willett WC. Health effects of trans-fatty acids: Experimental and observational evidence. European journal of clinical nutrition. 2009 May 2009;63(S2):S5-S21.

56. Fried SK, Rao SP. Sugars, hypertriglyceridemia, and cardiovascular disease. Am J Clin Nutr. 2003 October 2003;78(4):873S-80.

57. Cohen JC, Schall R. Reassessing the effects of simple carbohydrates on the serum triglyceride responses to fat meals. American journal of clinical nutrition. 1988 October 1988;48(4):1031-4.

58. Lairon D, Play B, Jourdheuil-Rahmani D. Digestible and indigestible carbohydrates: Interactions with postprandial lipid metabolism. Journal of nutritional biochemistry. 2007 April 2007;18(4):217-2.

59. Stanhope KL, Griffen SC, Bair BR, Swarbrick MM, Keim NL, Havel PJ. Twenty-four-hour endocrine and metabolic profiles following consumption of high-fructose corn syrup-, sucrose-, fructose-, and glucose-sweetened beverages with meals. American journal of clinical nutrition AJN. 2008 May 2008;87(5):1194-203.

60. Mekki N, Charbonnier M, Borel P, Leonardi J, Juhel C, Portugal H, et al. Butter differs from olive oil and sunflower oil in its effects on postprandial lipidemia and triacylglycerol-rich lipoproteins after single mixed meals in healthy young men. Journal of nutrition. 2002;132:3642-3649.

61. Sakr SW, Attia N, Haourigui M, Paul JL, Soni T, Vacher D, et al. Fatty acid composition of an oral load affects chylomicron size in human subjects. British journal of nutrition. 1997 January 1997;77(1):19-31.

62. Tholstrup T, Sandström B, Bysted A, Hølmer G. Effect of 6 dietary fatty acids on the postprandial lipid profile, plasma fatty acids, lipoprotein lipase, and cholesterol ester transfer activities in healthy young men. Am J Clin Nutr. 2001 February 2001;73(2):198-20.

63. Gatti E, Noe D, Pazzucconi F, Gianfranceschi G, Porrini M, Testolin G, et al. Differential effect of unsaturated oils and butter on blood glucose and insulin response to carbohydrate in normal volunteers. European journal of clinical nutrition. 1992 March 1992;46(3):161-6.

64. Oh K, Hu FB, Manson JE, Stampfer MJ, Willett WC. Dietary fat intake and risk of coronary heart disease in women: 20 years of follow-up of the nurses' health study. Am J Epidemiol. 2005 Apr 1, 2005;161(7):672-9.

List of Figures

Figure 1. X-ray diffraction patterns of sugar-free cookies (A) and sugar-free cakes (B) formulated with structured MAG gel (-), or MAG gel ingredients (- -).

Figure 2. Serum TAG response (A), AUC (B), peak value (C), and time-to-peak (D) over 6 h postprandial period following consumption of sugar-free cookies (SF-Cookie ◇) and cakes (SF-Cake ▲) prepared with the MAG gel, commercial recipe sugar cookies prepared with the MAG gel (S-Cookie-MAG ✳) and IE soy shortening (S-Cookie-IE ▼). Mean ± SEM, n=18. Different letters indicate significant difference between treatments, $P<0.05$.

Figure 3. Plasma glucose response (A), AUC (B), peak value (C), and time-to-peak (D) over 6 h postprandial period following consumption of sugar-free cookies (SF-Cookie ◇) and cakes (SF-Cake ▲) prepared with the MAG gel, commercial recipe sugar cookies prepared with the MAG gel (S-Cookie-MAG ✳) and IE soy shortening (S-Cookie-IE ▼). Mean ± SEM, n=18. Different letters indicate significant difference between treatments, $P<0.05$.

Figure 4. Serum insulin response (A), AUC (B), peak value (C), and time-to-peak (D) over 6 h postprandial period following consumption of sugar-free cookies (SF-Cookie ◇) and cakes (SF-Cake ▲) prepared with the MAG gel, commercial recipe sugar cookies prepared with the MAG gel (S-Cookie-MAG ✳) and IE soy shortening (S-Cookie-IE ▼). Mean ± SEM, n=18. Different letters indicate significant difference between treatments, $P<0.05$.

Figure 5. Serum FFA response (A), AUC (B), peak value (C), and time-to-peak (D) over 6 h postprandial period following consumption of sugar-free cookies (SF-Cookie ◇) and cakes (SF-Cake ▲) prepared with the MAG gel, commercial recipe sugar cookies prepared with the MAG gel (S-Cookie-MAG ✳) and IE soy shortening (S-Cookie-IE ▼). Mean ± SEM, n=18. Different letters indicate significant difference between treatments, $P<0.05$.

Figure 6. Correlations between postprandial glucose and insulin AUC values and available carbohydrate on a wet (A) and dry (B) weight basis for all cakes and cookies studied. Mean ± SEM, n=18

Figure 7. Postprandial TAG, glucose, and insulin AUC as a function of test meal available carbohydrate:fat, on both wet and dry weight bases.

Table 1– Dough formulations (wt %) for study treatments[1]

Ingredient	SF-Cookie-MAG	SF-Cookie-MAG gel ingredients	SF-Cake-MAG	SF-Cake-MAG gel ingredients	S-Cookie-MAG	S-Cookie-IE
MAG gel	33.64	-	33.53	-	22.78	-
MAG	-	1.51	-	1.51	-	-
Stearic acid	-	0.1	-	0.1	-	-
Water	4.59	4.58 (+13.44)[2]	36.56	36.52 (+ 13.40)[2]	-	7.98
NaCl	0.30	0.22	0.36	0.36	0.60	0.56
Sodium Bicarbonate	0.22	0.22	-	-	0.40	0.37
Flour	61.22	61.16	29.24	29.21	50.40	46.38
Oil	-	18.65	-	18.59	-	-
Baking powder	-	-	0.29	0.29	0.60	0.56
Sucrose	-	-	-	-	25.20	23.19
IE soy shortening	-	-	-	-	-	20.96
Total	100	100	100	100	100	100

[1] Products included sugar-free cakes and cookies with the structured MAG gel (SF-Cookie-MAG & SF-Cake-MAG), compositionally-equivalent MAG gel ingredients (SF-Cookie-MAG gel ingredients & SF-Cake-MAG gel ingredients) and commercial recipe sugar cookies containing the MAG gel (S-Cookie-MAG) or an industry standard interesterified soy shortening (S-Cookie-IE).

[2] Additional water included to account for water present in structured MAG gel.

Table 2- Weight, moisture, fat, available carbohydrate, and starch digestibility of baked study treatments[1,2]

	Weight (g)	Moisture (g)	Fat (g)	Energy per serving (kcal)	Energy from macronutrients (%)	Available Carbohydrate (g)	Starch Digestibility[2,3]
SF-Cookie-MAG	157.6	2.7	40.4	794.2	CHO- 49.3 Fat- 45.7 Protein- 4.9	89.6	52.5 ± 2.3 - RDS[a] 46.3 ± 3.6 - SDS[a] 1.1 - ResS
SF-Cookie-MAG gel ingredients	164.2	2.3	40.3	793.4	CHO- 49.3 Fat- 45.7 Protein- 4.9	94.8	57.5 ± 2.0 - RDS[b] 42.4 ± 4.3 - SDS[a] 0.3 – ResS
SF-Cake-MAG	158.7	66.5	40.2	572.1	CHO- 33.4 Fat- 63.2 Protein- 3.2	41.4	78.9 ± 1.4 - RDS[c] 23.8 ± 2.5 - SDS[c] 0.0 – ResS
SF-Cake-MAG gel ingredients	160.1	67.1	40.2	569.8	CHO- 33.2 Fat- 63.4 Protein- 3.2	43.9	68.3 ± 0.4 - RDS[d] 24.5 ± 3.0 - SDS[c] 7.2 – ResS
S-Cookie-MAG	187.1	1.9	27.5	801.6	CHO- 65.1 Fat- 30.8 Protein- 4.0	123.9	51.3 ± 0.0 - RDS[a] 48.7 ± 0.1 - SDS[a] 0.0 – ResS
S-Cookie-IE	191.2	3.3	42.1	892.5	CHO- 54.2 Fat- 42.4 Protein- 3.3	116.9	48.9 ± 3.0 - RDS[a] 50.3 ± 5.8 - SDS[a] 0.8 – ResS

[1] Mean ± SD, n = 3.

[2] Different superscript letters a-d between values within each fraction indicates $P<0.05$.

[3] RDS, SDS, and ResS indicate rapidly digestible starch, slowly digestible starch and residual starch, % total starch on a dry weight basis).

Table 3 –Participant characteristics[1, 2]

Characteristic	
Age (y)	23.6 ± 0.7
Height (cm)	181.3 ± 2.2
Body Weight (kg)	76.7 ± 1.8
BMI (kg/m^2)	23.4 ± 0.5
Systolic Blood Pressure (mmHg)	130.9 ± 2.2
Diastolic Blood Pressure (mmHg)	76.9 ± 1.6
Body Fat (%)	14.4 ± 2.2
Fasting Plasma Glucose (mmol/L)	4.9 ± 0.1
Fasting Serum Insulin (pmol/L)	22.4 ± 2.9
Fasting Triacylglycerol (mmol/L)	1.0 ± 0.1
Fasting Total Cholesterol (mmol/L)	4.0 ± 0.2
Fasting HDL Cholesterol (mmol/L)	1.2 ± 0.1
Fasting LDL Cholesterol (mmol/L)	2.4 ± 0.2
Fasting Total/HDL Cholesterol	3.6 ± 0.2

[1] Mean ± SEM, n=18.
[2] As determined at fasting on first study visit.

Table 4 -Summary of contrast testing for study main effects[1]

Comparison	TAG	Glucose	Insulin	FFA
MAG gel cookies vs. cakes	AUC[2] Peak value[2]	AUC[2] Peak value[3] Time-to-peak[3]	AUC[2] Peak value[2] Time-to-peak[3]	Not significant
Effect of MAG gel structure (i.e. MAG gel vs. compositionally equivalent ingredients) in sugar free products	Not significant	Not significant	Not significant	Not significant
Did effect of structure differ between cookies & cakes?	Not significant	Not significant	Not significant	Not significant
Sugar cookie comparison: MAG gel vs. IE soy shortening	Not significant	Not significant	Not significant	Not significant
Sugar cookie with MAG gel vs. Sugar-free Cookie with MAG gel	Not significant	Peak value[3] Time-to-peak[4]	AUC[5] Peak value[3] Time-to-peak[3]	Not significant

[1] Unless otherwise indicated, $P<0.05$.
[2] $P<0.001$
[3] $P<0.01$
[4] $P=0.056$
[5] $P=0.086$

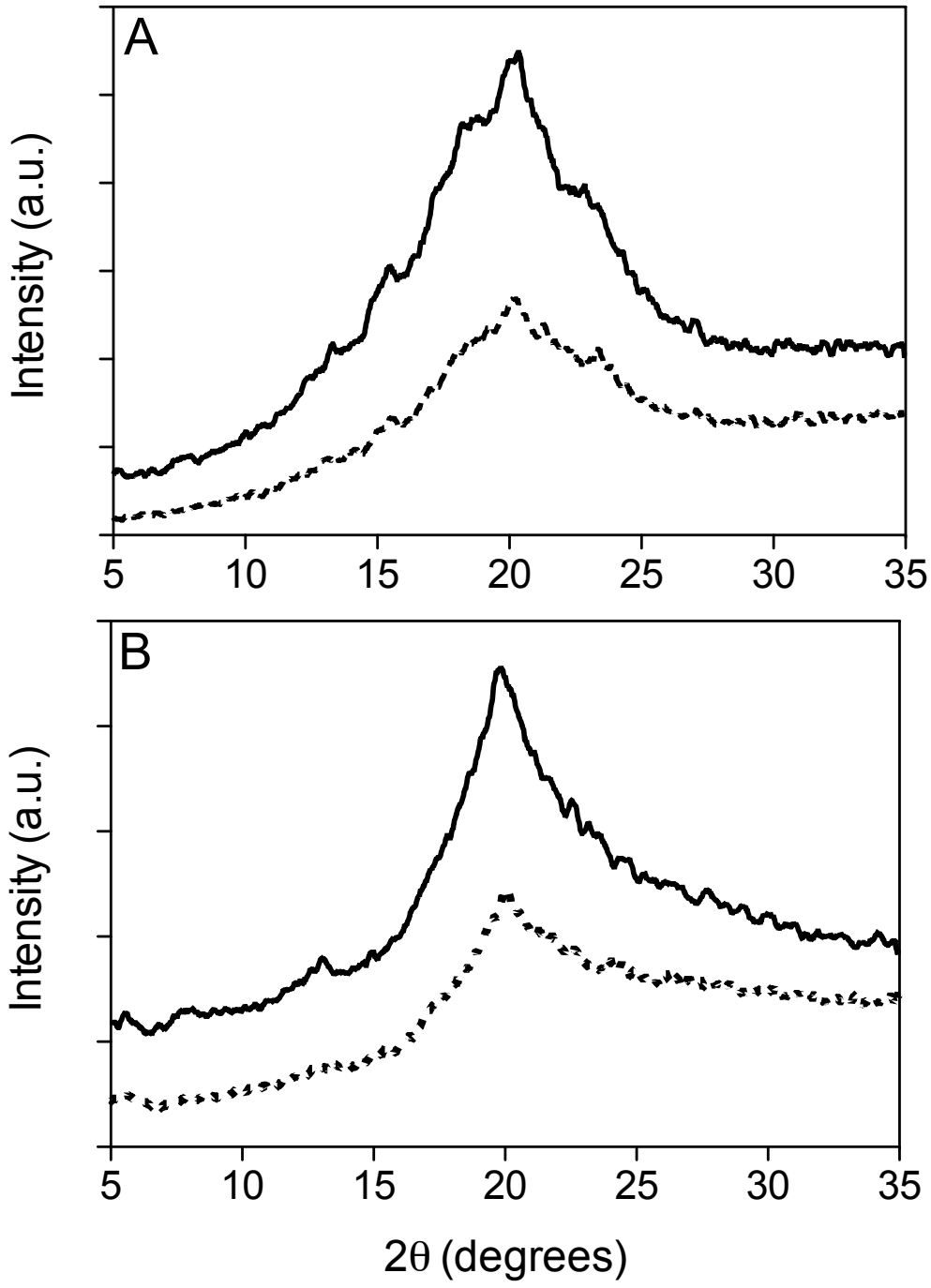

Figure 1. X-ray diffraction patterns of sugar-free cookies (A) and sugar-free cakes (B) formulated with structured MAG gel (-), or MAG gel ingredients (- -).

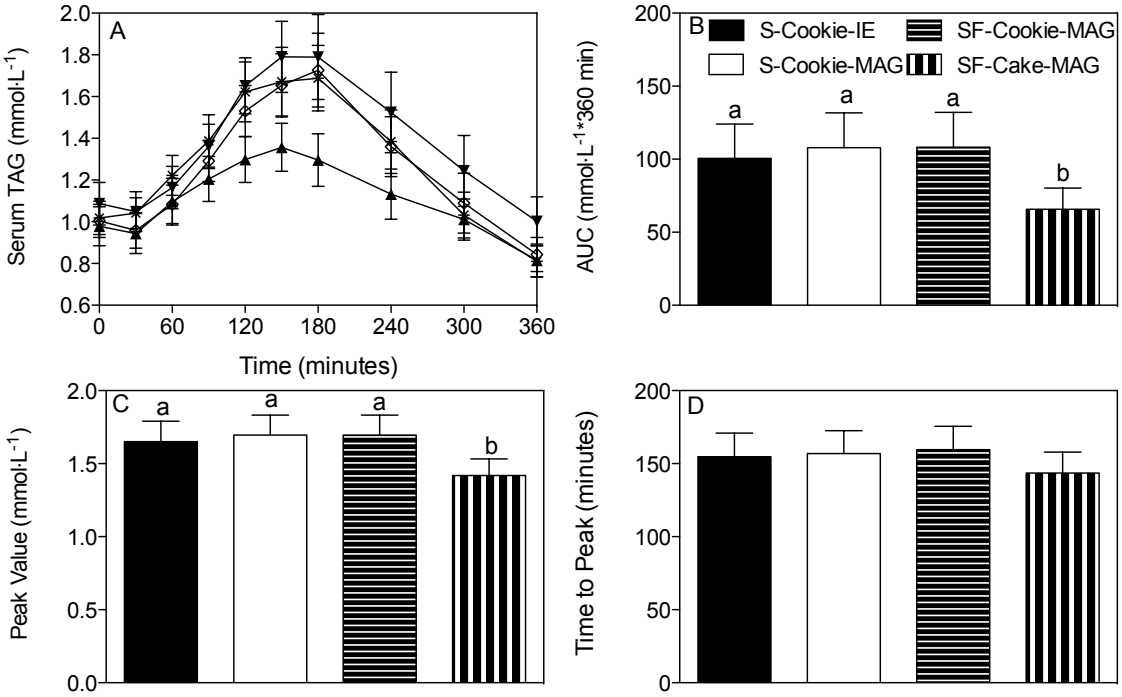

Figure 2 – Serum TAG response (A), AUC (B), peak value (C), and time-to-peak (D) over 6 h postprandial period following consumption of sugar-free cookies (SF-Cookie ◇) and cakes (SF-Cake ▲) prepared with the MAG gel, commercial recipe sugar cookies prepared with the MAG gel (S-Cookie-MAG ✳) and IE soy shortening (S-Cookie-IE ▼). Mean ± SEM, n=18. Different letters indicate significant difference between treatments, $P<0.05$.

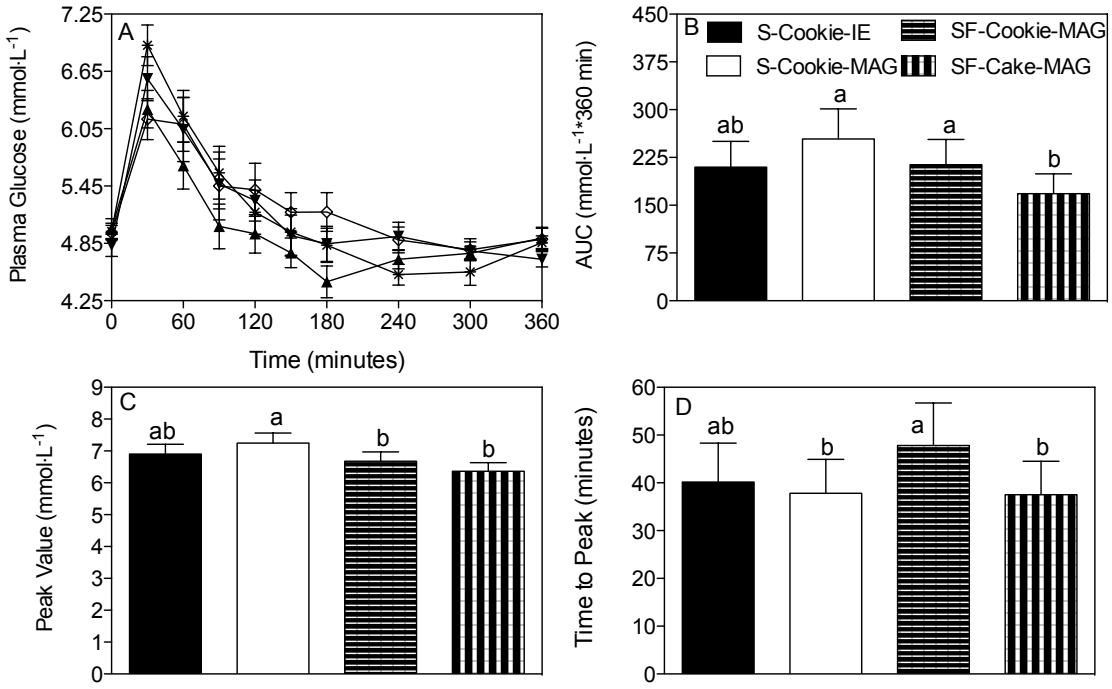

Figure 3 – Plasma glucose response (A), AUC (B), peak value (C), and time-to-peak (D) over 6 h postprandial period following consumption of sugar-free cookies (SF-Cookie ◇) and cakes (SF-Cake ▲) prepared with the MAG gel, commercial recipe sugar cookies prepared with the MAG gel (S-Cookie-MAG ✳) and IE soy shortening (S-Cookie-IE ▼). Mean ± SEM, n=18. Different letters indicate significant difference between treatments, $P<0.05$.

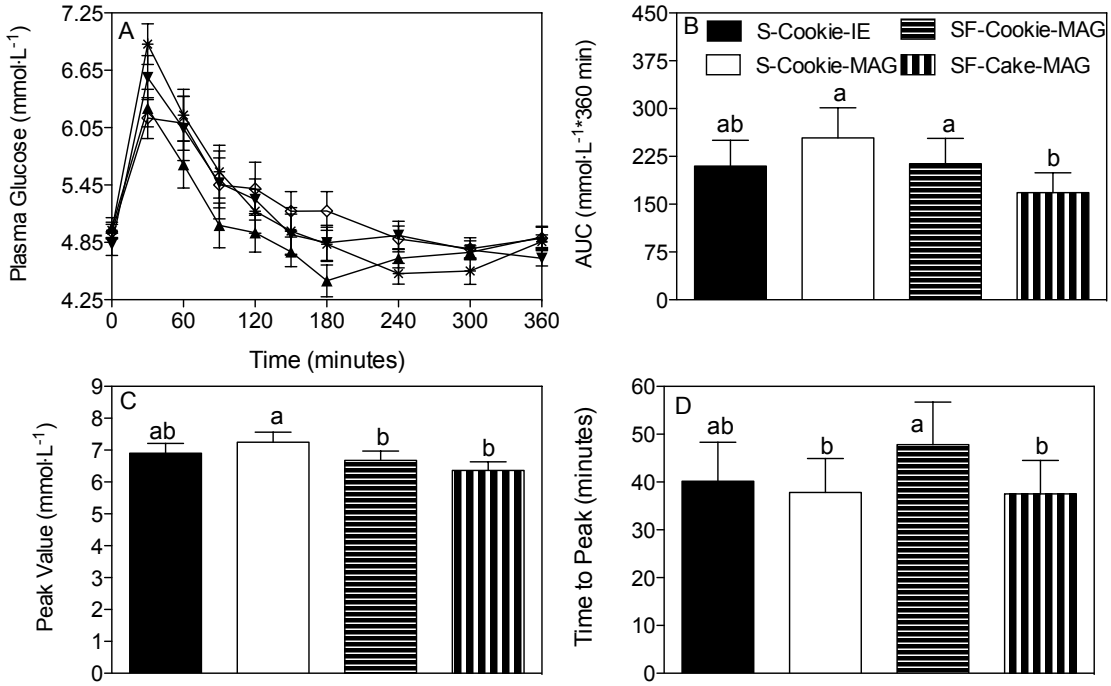

Figure 4 – Serum insulin response (A), AUC (B), peak value (C), and time-to-peak (D) over 6 h postprandial period following consumption of sugar-free cookies (SF-Cookie ◇) and cakes (SF-Cake ▲) prepared with the MAG gel, commercial recipe sugar cookies prepared with the MAG gel (S-Cookie-MAG ✳) and IE soy shortening (S-Cookie-IE ▼). Mean ± SEM, n=18. Different letters indicate significant difference between treatments, $P<0.05$.

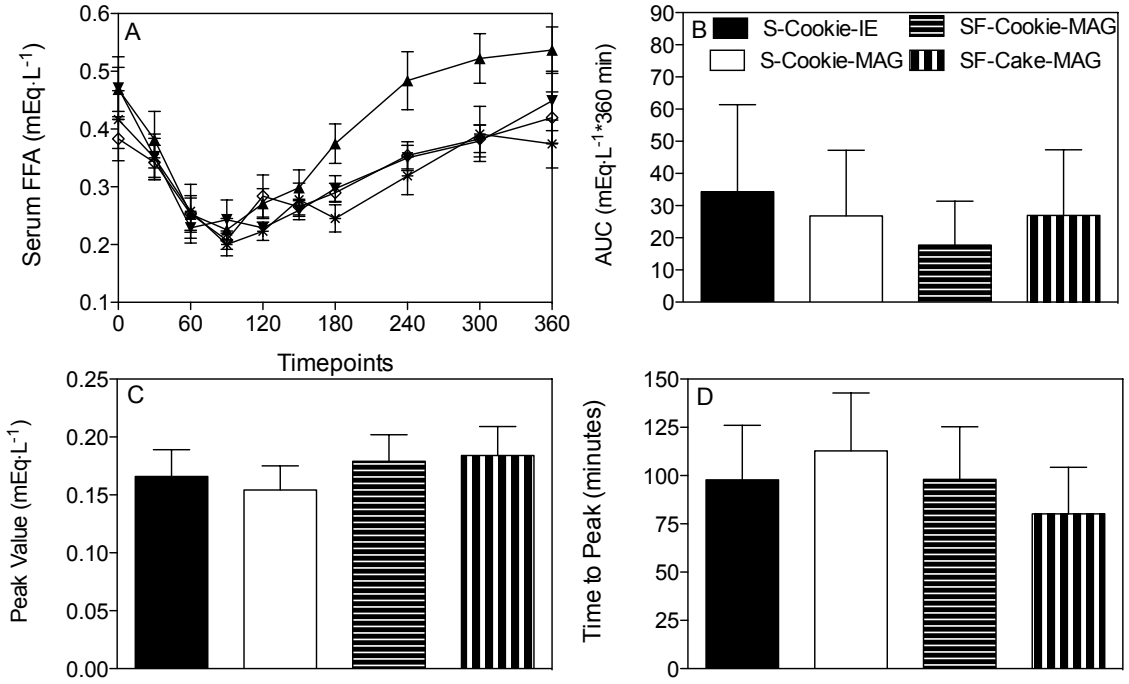

Figure 5 – Serum FFA response (A), AUC (B), peak value (C), and time-to-peak (D) over 6 h postprandial period following consumption of sugar-free cookies (SF-Cookie ◇) and cakes (SF-Cake ▲) prepared with the MAG gel, commercial recipe sugar cookies prepared with the MAG gel (S-Cookie-MAG ✳) and IE soy shortening (S-Cookie-IE ▼). Mean ± SEM, n=18. Different letters indicate significant difference between treatments, $P<0.05$.

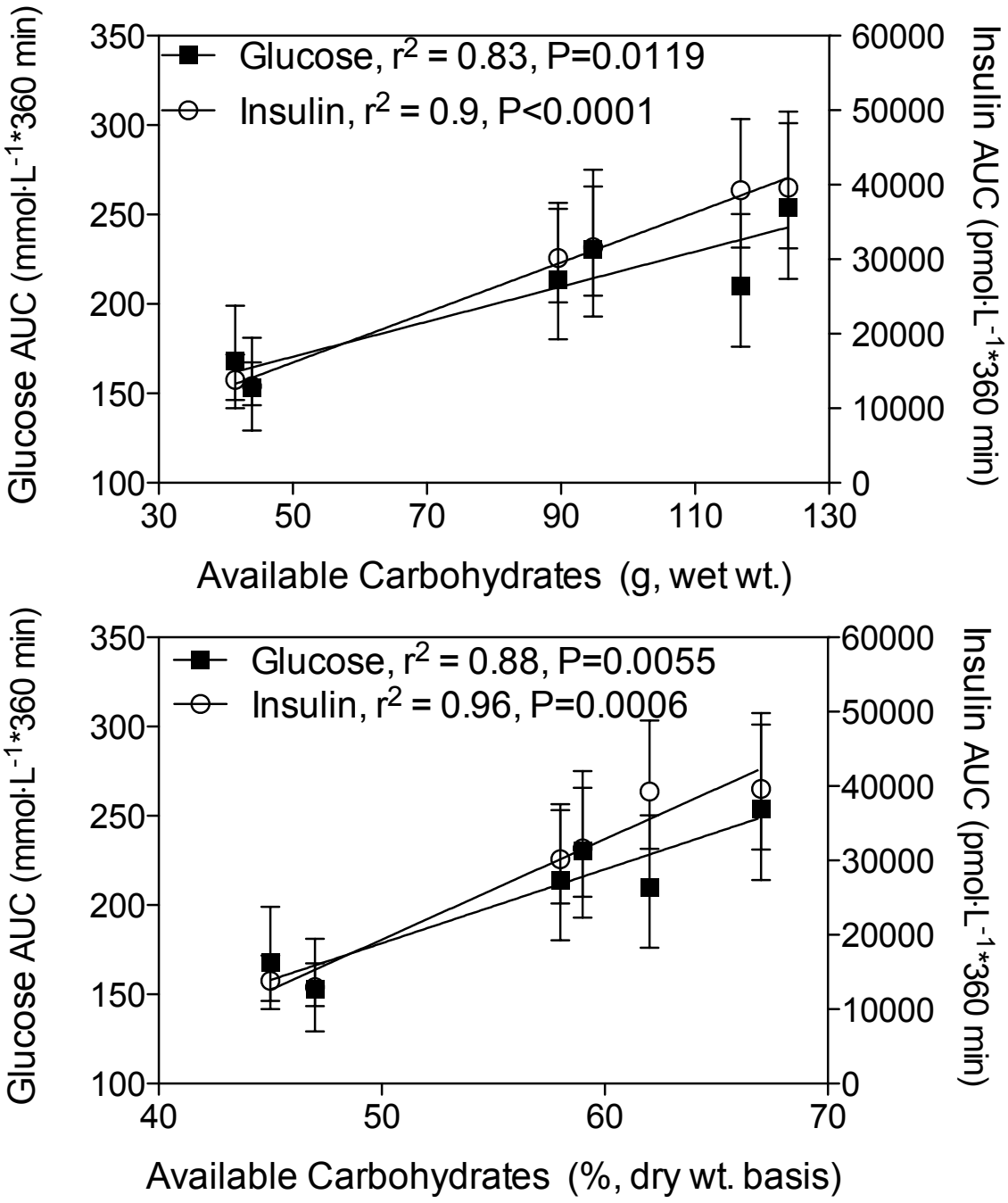

Figure 6 – Correlations between postprandial glucose and insulin AUC values and available carbohydrate on a wet (A) and dry (B) weight basis for all cakes and cookies studied. Mean ± SEM, n=18.

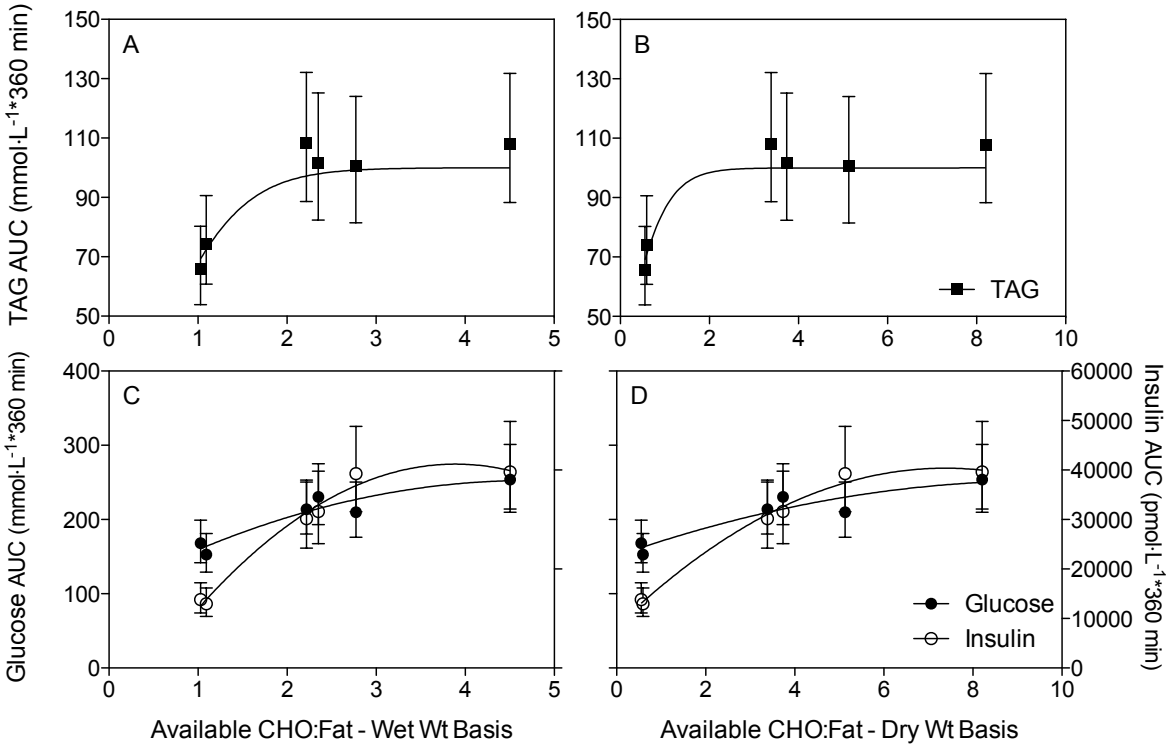

Figure 7. Postprandial TAG, glucose, and insulin AUC as a function of test meal available carbohydrate:fat, on both wet and dry weight bases.