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Food Fried in Extra-Virgin Olive Oil Improves Postprandial Insulin Response in Obese, Insulin-Resistant Women

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ABSTRACT The benefits of low glycemic load (GL) diets on clinical outcome in several metabolic and cardiovascular diseases have extensively been demonstrated. The GL of a meal can be affected by modulating the bioavailability of carbohydrates or by changing food preparation. We investigated the effect on plasma glucose and insulin response in lean and obese women of adding raw or fried extra-virgin olive oil to a carbohydrate-containing meal. After an overnight fast, 12 obese insulin-resistant women (body mass index [BMI], $32.8 \pm 2.2\,kg/m^2$) and five lean subjects (BMI, $22.2 \pm 1.2\,kg/m^2$) were randomly assigned to receive two different meals (designated A and B). Meal A was composed of 60 g of pasta made from wheat flour and 150 g of grilled courgettes with 25 g of uncooked oil. Meal B included 15 g of oil in the 150 g of deep-fried courgettes and 10 g of oil in the 60 g of stir-fried pasta. Both meals included 150 g of apple. Blood samples were collected at baseline and every 30 minutes over a 3-hour post-meal period and were tested for levels of glucose, insulin, C-peptide, and triglycerides. The area under the curve (AUC) values were calculated. In obese women the AUCs for C-peptide were significantly higher after meal A than after meal B at 120 minutes (W [Wilcoxon sign rank test] = 27.5, $P = .0020$), 150 minutes ($W = 26.5$, $P = .0039$), and 180 minutes ($W = 26.5$, $P = .0039$). No differences were found in lean subjects. This study demonstrated that in obese, insulin-resistant women, food fried in extra-virgin olive oil significantly reduced both insulin and C-peptide responses after a meal.

KEY WORDS: • ***C-peptide response*** • ***cooking methods*** • ***extra-virgin olive oil*** • ***fried food*** • ***glycemic load*** • ***insulin response*** • ***monounsaturated fatty acids*** • ***obese, insulin-resistant women***

INTRODUCTION

The therapeutic and preventive roles of low glycemic index (GI) and low glycemic load (GL) diets have been extensively examined[1] and have become a matter of international attention because of the World Health Organization's recommendations to reduce the overall dietary consumption of sugars and rapidly digestible starches.[2] Consumption of a low GL regular-fat diet plays an important role in diseases associated with high circulating insulin levels, such as type 2 diabetes mellitus,[3] obesity,[4] cardiovascular disease, and cancer. The GL of a meal depends on its overall fat and protein content,[5] on how it is cooked,[6] and on the level of processing of the food. Several studies have reported that the addition of fat improves glycemic response to carbohydrate foods[7] because of different mechanisms, including increase of the viscosity of intestinal contents, delay in gastric emptying,[8] and processes mediated by incretin hormones.[9] Furthermore, other studies failed to demonstrate any effect on glycemic control with the co-ingestion of fat[10] and demonstrated no difference with different degrees of fat saturation.[7] A possible explanation may involve inter-individual variability among the different studies because the more severe the glycemic deregulation is, the greater the positive effect of reducing GL is,[11] including by variation of fat content. Because it is reasonable to consider GL as a predictor of carbohydrate effect on health, it should be taken into account that this is a concept based on the consumption of a single food by itself. In reality, it is rarely the case that we ingest a single food alone, but more likely the food is ingested in association with other foods. In this case, the glycemic response cannot be predicted from the total GL value of a single food because of the differences in energy, protein, and fat content of a mixed meal.[12] On the other hand, it is necessary to identify the factors that influence the GL of a meal, in order to provide simple dietary instructions to patients and consumers.[9]

Therefore, the aim of the present study was to investigate, in lean and obese insulin-resistant women, the effect of the

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addition to a carbohydrate-containing meal of either uncooked or fried extra-virgin olive oil on plasma glucose levels and insulin response.

SUBJECTS AND METHODS

Subjects

Out of a sample of 183 patients admitted to the outpatient Clinic of Metabolic Diseases at the Catholic University in Rome, Italy, between January 2007 and October 2008, 12 obese insulin-resistant nondiabetic women (41 ± 4 years old, body mass index [BMI] $32.8 \pm 2.2\,kg/m^2$) were enrolled in the study. A group of five healthy lean women (43 ± 2 years old, BMI $22.2 \pm 1.2\,kg/m^2$) was recruited to participate in the study as control subjetcs.

Exclusion criteria were as follows: endocrine disorders, use of drugs that could influence data collection, liver or renal diseases, fever, pregnancy, high number of cigarettes smoked daily (more than 10), and intensive physical activity. All the subjects were evaluated in the follicular phase of the menstrual cycle and underwent a physical examination and laboratory analysis, including an oral glucose tolerance test, within 3 months of enrollment in the study to rule out diabetes mellitus. In particular, all the subjects enrolled had a glucose level at baseline of $<126\,mg/dL$ and at 120 minutes postload of $<200\,mg/dL$.

All anthropometric measurements were done with the subjects in the fasting state. BMI was calculated as weight (in kg)/height (in m^2). Body composition, *i.e.*, fat mass and fat-free mass, were assessed by dual-energy X-ray absorptiometry, using a whole-body densitometer (Lunar Prodigy Advance, GE Healthcare, Little Chalfont, UK) with Encore 2005 software.

The anthropometric characteristics, homeostasis model assessment of insulin resistance (HOMA-IR), and hematochemical parameters of the two groups are reported in Table 1. T1 ▶

Table 1. Baseline Anthropometric, Body Composition Variables, and Hematochemical Values of the Subjects Examined

	Obese subjects (n = 12)	*Lean subjects (n = 5)*
Age (years)	41 ± 4	43 ± 2
Waist (cm)	102 ± 3*	76 ± 3
BMI (kg/m^2)	32.8 ± 2.2*	22.2 ± 1.2
Fat mass (%)	32.0 ± 3.9*	22.1 ± 1.4
HOMA-IR	2.8 ± 1.4*	1.7 ± 1.2
Uric acid (mg/dL)	4.25 ± 0.60*	3.65 ± 0.70
Total cholesterol (mg/dL)	201 ± 42	196 ± 54
LDL-C (mg/dL)	97.4 ± 35.3	112.7 ± 45.3
HDL-C (mg/dL)	54.8 ± 8.7*	72.0 ± 8.2
GPT (IU/L)	24.3 ± 13.5	13.7 ± 6.8
Triglycerides (mg/dL)	106.6 ± 33.6*	58.1 ± 14.4
P-Cholinesterase (IU/L)	$7{,}173 \pm 1165$	$6{,}647 \pm 1283$

Data are are mean ± SD values.

*$P < .01$.

BMI, body mass index; GPT, glutamic-pyruvic transaminase; HDL-C, high-density lipoprotein-cholesterol; HOMA-IR, homeostasis model assessment of insulin resistance; LDL-C, low-density lipoprotein-cholesterol.

Experimental design

The day before the testing day, subjects were asked to avoid any strenuous exercise and to eat a standardized dinner containing 60% carbohydrates, 25% fat, and 15% protein. Capillary blood samples for glucose, insulin, and C-peptide were collected at baseline and every 30 minutes over a 3-hour postprandial period. Blood samples were collected in an ice bath, immediately separated by centrifugation at 4°C, and stored at −80°C until assay. Plasma glucose was measured by the glucose oxidase method (Beckman, Fullerton, CA, USA). Plasma insulin was assayed by microparticle enzyme immunoassay (Abbott, Pasadena, CA, USA) with a sensitivity of $1\,\mu U/mL$ and an intra-assay coefficient of variation of 6.6%. C-peptide was assayed by radioimmunoassay (MYRIA, Technogenetics, Milan, Italy); this assay has a minimal detectable concentration of 17 pmol/L and inter- and intra-assay coefficients of variation of 3.3–5.7% and 4.6–5.3%, respectively. HOMA-IR was calculated from fasting glucose and insulin levels using the following equation: (insulin [in $\mu U/mL$] glycemia [in mmol/L])/22.5.

Capillary blood samples for other hematochemical parameters were also collected at baseline. Total cholesterol, triglycerides, and uric acid were determined by enzymatic-colorimetric methods. High-density lipoprotein (HDL)-cholesterol (HDL-C) was determined after selective precipitation of non-HDL fractions by dextran sulfate-magnesium chloride by an enzymatic-colorimetric method. Low-density lipoprotein-cholesterol (LDL-C) was calculated by the formula of Friedewald *et al.*:[13] total cholesterol – (triglycerides/5 + HDL-C) for triglycerides $<400\,mg/dL$. Glutamic-pyruvic transaminase activity was assayed by enzymatic methods, and that of P-cholinesterase by a colorimetric method.

Triglycerides were measured at baseline and at 180 minutes after consumption of the experimental meals.

Experimental meals

Two experimental meals were administered to the subjects in a random order in which each subject provided her own control. The meals were served at the same time in the morning and were given 1 week apart. Each meal, containing 66.2 g of carbohydrates and 32.1 g of lipids, from extra-virgin olive oil, was consumed over a 15-minute period and served with 200 mL of water. The macronutrient composition of the two test meals was calculated using the Dietosystem (Milan) software (2002); data are given in Table 2. Test meal A consisted of 60 g of penne pasta cooked in boiling, unsalted water for 10 minutes, as indicated by the manufacturer, served with 10 g of raw extra-virgin olive oil, 150 g of courgettes (cut in slices of the same size, grilled for 4 minutes each face, and dressed with 15 g of raw extra-virgin olive oil), and 150 g of apple. Test meal B consisted of the same amount of penne pasta stir-fried in a ◀ T2

Table 2. Macronutrient Composition of the Test Meals

Macronutrient	*Composition*
Energy (kcal)	572.43
Carbohydrates (g)	66.21
Starch	41.01
Sugars	21.12
Lipids (g)	32.11
Saturated	5.17
Monounsaturated	23.19
Polyunsaturated	3.21
Protein (g)	8.79
Fiber (g)	7.47
Soluble	1.79
Insoluble	3.69

pan for 15 seconds with 10 g of extra-virgin olive oil fried for 3 minutes, 150 g of courgettes deep fried in extra-virgin olive oil, and 150 g of apple. The retained oil of the fried courgettes was calculated to be 15 g.

The penne pasta was purchased from a Rome retail market. Courgettes, of approximately the same size each, and yellow apples were bought from a local farmer.

Ethics

Approval of the experimental protocol was given by the Ethics Committee of the Catholic University of Rome, and all subjects gave their written informed consent to participate in the study.

Statistical analysis

For each subject, the impact of both meals A and B was calculated by plotting three pairs of curves showing the response of plasma glucose, plasma insulin, and plasma C-peptide at 30, 60, 120, 150, and 180 minutes after administration of the experimental meals. The integrated area under the curve (AUC) for each curve was calculated by the trapezoidal method for three time intervals, at 120, 150 and 180 minutes, respectively, after the experimental meal. A Wilcoxon sign rank test (W) was applied in order to reveal any statistical difference in the effect of the experimental meals in both the lean and obese groups of participants. Although all AUCs consisted of ordered data, the above non-parametric estimator was chosen after examining the AUC distribution, which suggested a violation of the assumed normality required for parametric methods. A value of $P < .05$ was considered significant enough to dismiss the null hypothesis that no difference existed in the effect between meals A and B. All analyses were conducted with the statistical software package SAS version 9.2 (SAS Institute, Cary, NC, USA).

RESULTS AND DISCUSSION

The modulation of the GL of a meal plays a pivotal role in the treatment of type 2 diabetes mellitus and obesity, and few data are available in the literature on the effect of different food preparation and combination on glucose and insulin response in humans.[14]

Previous studies demonstrated that the addition of fat to a meal is able to reduce the GI of the food and to decrease circulating insulin levels.[15] The degree of fat saturation showed no effect on postprandial hyperglycemia, insulinemic response, or satiety in healthy men,[8] while the substitution of saturated fatty acids for monounsaturated fatty acids (MUFAs) had favorable effects on serum lipid profiles and risk of developing coronary artery disease.[16]

In the present study we found that obese subjects had higher body weight, larger waist circumference, higher BMI, higher uric acid and triglycerides, and lower HDL-C levels with respect to lean subjects, as shown in Table 1. All the obese subjects had a mean HOMA-IR of 2.8 ± 1.4, indicating an insulin-resistant state.

In meal B, in lean healthy women, fried extra-virgin olive oil showed no biological effect on the glycemic and insulinemic response. These findings could be attributed, as previously reported, to the fact that the more severe the glycemic deregulation, the greater the positive effect of the addition of fat to high GI food on glycemic metabolism.[11] In contrast, meal B reduced the insulin response and circulating C-peptide concentrations in obese women. No statistical differences were found in plasma glucose concentrations calculated from AUCs at 120, 150, or 180 minutes after meal A or B ingestion ($W = 5.5$, $P = .625$; $W = 2.5$, $P = .845$; and $W = 3.5$, $P = .7695$, respectively). Insulin response at 120 ($W = 27.5$, $P = .0020$) and 150 ($W = 25.5$, $P = .0059$) minutes was significantly higher after meal A rather than meal B in obese women, whereas no statistical difference was observed at 180 minutes ($W = 17.5$, $P = .0840$) (Fig. 1). ◀F1

The AUCs for C-peptide were significantly higher after meal A than after meal B at 120 ($W = 27.5$; $P = .0020$), 150 ($W = 26.5$, $P = .0039$), and 180 ($W = 26.5$, $P = .0039$) minutes in obese women (Fig. 1). No statistical differences were found in blood glucose, insulin, and C-peptide values after both meals A and B in lean subjects (Fig. 1).

The triglyceride concentration (in mg/dL) did not vary between meals A and B in lean subjects (meal A, baseline 58.1 ± 14.4 and at 180 minutes 108 ± 26.2; meal B, baseline 60.2 ± 18.4 and at 180 minutes 110.3 ± 28.5), while a non-statistically significant trend towards higher levels of triglyceride after meal A was found in obese women (meal A, baseline 106.6 ± 36.2 and at 180 minutes 193.5 ± 41.2; meal B, baseline 95.4 ± 28.6 and at 180 minutes 175.4 ± 42.3). This could be due to decreased insulin response after meal B ingestion. Insulin secretion, in fact, is known to stimulate hepatic production of very-low-density lipoprotein, thus increasing postprandial circulating triglyceride levels.[4]

The frying process is known to generate the formation of resistant starches, in foods such as potato products and stir-fried food such as pasta, by generating the formation of amylose lipid complexes. These complexes are slowly digested by α-amylase, which has been demonstrated both *in vivo* and *in vitro*.[17] Therefore, if the frying process is carried out with careful control of cooking temperature and

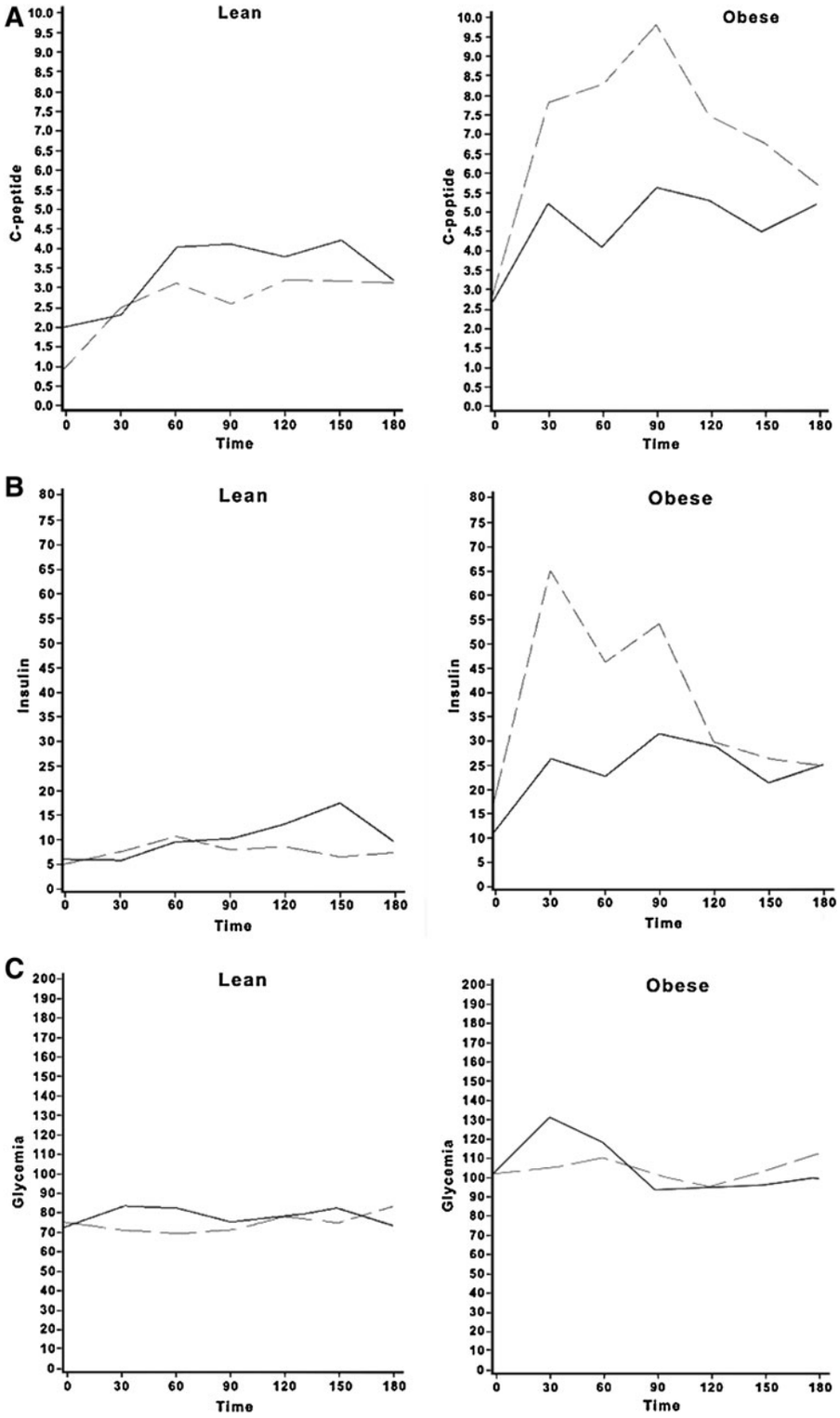

FIG. 1. A typical dynamic response (**right panels**) in an obese patient and (**left panels**) in a lean patient tested after meal B (continuous line) and test meal A (broken line): circulating levels of (**A**) C-peptide, (**B**) insulin, and (**C**) glucose.

time, in order to avoid the production of toxic substances[18,19] fried food could be more useful for reduction of carbohydrate absorption rate than the simple addition of raw fats.

The finding that the glycemic response rate did not vary significantly in both obese and lean women after consumption of meal B of stir-fried pasta with deep-fried courgettes could be due to meal B containing a relatively small amount of fried carbohydrates, which were not capable of inducing significant variations in plasma glucose concentrations. It has been well demonstrated that deep-fat frying, because of the higher formation of free fatty acids, shows a greater choleretic and cholagogue action compared to raw oil.[20] These free fatty acids cause a contraction and decongestion of the gallbladder after the meal. Therefore, in the present study, it could be assumed that the reduction in insulin secretion after meal B in the obese insulin-resistant subjects is explained not only by the lower glucose absorption *per se*, but by the use of fried oil in stir-fried pasta and deep-fried courgettes. This could have had an effect on the hepatic regulation of glucose and insulin metabolism. Further studies with a larger-sized sample and an accurate monitoring of liver function are needed to clarify this point.

Therefore deep-fat frying could improve insulin metabolism and, by favoring bile excretion by the small bowel, could facilitate detoxifying mechanisms of the liver. Previous studies suggested that diets higher in MUFAs improved insulin sensitivity,[21,22] by the mediation of incretin hormones, such as gastric inhibitory polypeptide, glucagon-like peptide 1, and cholecystokinin.[23] The mechanism of action of fried extra-virgin olive oil could be associated with its thermal stability and the release of MUFAs when cooked.

In the present study, only women were enrolled, because of the higher percentage of female subjects admitted to our center, but further studies including men are warranted to support our findings in both sexes. Because it is a preliminary study and the protocol required a great compliance by both patients and control subjects, only 12 obese patients and five lean subjects were examined. A wider range of subjects is warranted to better support our hypothesis.

In conclusion, this preliminary study investigated for the first time the effect of food fried in extra-virgin oil on glucose and insulin response in humans. In our study we found that in obese insulin-resistant women, food fried in extra-virgin olive oil significantly reduced both insulin and C-peptide responses after a mixed meal. No differences were found in lean subjects. Future studies utilizing a greater carbohydrate load and with a larger number of subjects are needed to better clarify the mechanism of the frying process in reducing the insulin response to a meal.

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AUTHOR DISCLOSURE STATEMENT

No competing financial interests exist.

REFERENCES

1. Shai I, Schwarzfuchs D, Henkin Y, *et al.*: Weight loss with a low-carbohydrate, Mediterranean, or low-fat diet. *N Engl J Med* 2008;359:229–241.
2. Adam-Perrot A, Clifton P, Brouns F: Low-carbohydrate diets: nutritional and physiological aspects. *Obes Rev* 2006;7:49–58.
3. Hu FB, Van Dam RM, Liu S: Diet and risk of type II diabetes: the roles of types of fat and carbohydrate. *Diabetologia* 2001;44: 805–817.
4. Venn BJ, Green TJ: Glycemic index and glycemic load: measurement issues and their effect on diet-disease relationship. *Eur J Clin Nutr* 2007;61(Suppl 3):122–131.
5. Henry CJK, Lightowler HJ, Kendall FL, Storey M: The impact of the addition of toppings/fillings on the glycaemic response to commonly consumed carbohydrate foods. *Eur J Clin Nutr* 2006; 60:763–769.
6. Najjar N, Adra N, Hwalla N: Glycemic and insulinemic responses to hot vs cooled potato in males with varied insulin sensivity. *Nutr Res* 2004;24:993–1004.
7. Lunetta M, Di Mauro M, Crimi S, Mughini L: Influence of different cooking processes on the glycaemic responses to potatoes in non-insulin dependent diabetic patients. *Diabetes Nutr Metab* 1995;8:49–53.
8. MacIntosh CG, Holt SHA, Brand-Miller JC: The degree of fat saturation does not alter glycemic, insulinemic or satiety responses to as starchy staple in healthy men. *J Nutr* 2003;133: 2577–2580.
9. Thomsen C, Rasmussen O, Lousen T, *et al.*: Differential effects of saturated and monounsaturated fatty acids on postprandial lipemia and incretin response in healthy subjects. *Am J Clin Nutr* 1999;69:1135–1143.
10. Leeman M, Ostman E, Bjorck I: Glycaemic and satiating properties of potato products. *Eur J Clin Nutr* 2008;62:87–95.
11. Howlett J, Ashwell M: Glycemic response and health: summary of a workshop. *Am J Clin Nutr* 2008;87(Suppl 1):212–216.
12. Flint A, Moller BK, Raben A, *et al.*: The use of the glycaemic index tables to predict glycaemic index of composite breakfast meals. *Br J Nutr* 2004;91:979–989.
13. 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; 18:499–502.
14. Mann J, Cummings JH, Englyst HN, *et al.*: FAO/WHO scientific update on carbohydrates in human nutrition: conclusions. *Eur J Clin Nutr* 2007;61(Suppl 1):132–137.
15. Galgani J, Aguirre C, Díaz E: Acute effect of meal glycemic index and glycemic load on blood glucose and insulin responses in humans. *Nutr J* 2006;5:22.
16. Hu FB, Manson JE, Willett WC: Types of dietary fat and risk of coronary heart disease: a critical review. *J Am Coll Nutr* 2001; 20:5–19.
17. Tufvesson F, Skrabanja V, Björck I, Elmståhl HL, Eliasson AC: Digestibility of starch systems containing amylose-glycerol monopalmitin complexes. *Food Sci Technol Lebens Wissen Technol* 2001;34:131–139.

18. Fott RJ, Haase NH, Grob K, Gondè P: Acrylamide in fried roasted products: a review on progress in mitigation. *Food Add Cont* 2007;24(Suppl 1):37–46.
19. Choe E, Min DB: Chemistry of deep-fat frying oils. *J Food Sci* 2007;72:R77–R86.
20. Shiratori K, Watanabe S, Chey WY, Lee KY, Chang TM: Endogenous cholecystokinin drives gallbladder emptying in dogs. *Am J Physiol* 1986;251:G553–G558.
21. Vessby B, Unsitupa M, Hermansen K, Riccardi G, Rivellese AA, Tapsell LC: Substituting dietary saturated for monounsaturated fat impairs insulin sensitivity in healthy men and women: the KANWU Study. *Diabetologia* 2001;44:312–319.
22. Riccardi G, Rivellese AA, Giacco R: Role of glycemic index and glycemic load in the healthy state, in prediabetes, and in diabetes. *Am J Clin Nutr* 2008;87:269S–274S.
23. Fried M, Schwizer W, Beglinger C, Keller U, Jansen JB, Lamers CB: Physiological role of cholecystokinin on postprandial insulin secretion and gastric meal emptying in man. Studies with the cholecystokinin receptor antagonist loxiglumide. *Diabetologia* 1991;34:721–726.