Meal-Induced Oxidative Stress and Low-Density Lipoprotein Oxidation in Diabetes: The Possible Role of Hyperglycemia

Antonio Ceriello, Nadia Bortolotti, Enrico Motz, Carlo Pieri, Michele Marra, Laura Tonutti, Sebastiano Lizzio, Fabiola Feletto, Barbara Catone, and Claudio Taboga

Oxidative stress and its contribution to low-density lipoprotein (LDL) oxidation have been implicated in the pathogenesis of vascular diabetic complications. However, the relationship between hyperglycemia, hyperinsulinemia, hyperlipidemia, and oxidative stress is still debated. If plasma glucose and/or insulin and/or lipid are some of the most important determinants of oxidative stress in diabetes, then their typical postprandial elevations in diabetes would be expected to favor oxidative stress and LDL oxidation. To test this hypothesis, in type 2 diabetic patients, we evaluated the effects of two different standard meals designed to produce different levels of postprandial hyperglycemia on the plasma oxidative status and LDL oxidation. The meals were administered in randomized order to each of 10 type 2 diabetic patients. Blood samples were collected at baseline and 60 and 120 minutes after the meals. In every sample, plasma levels of glucose, insulin, cholesterol, triglycerides, nonesterified fatty acids (NEFAs), malondialdehyde (MDA), and the total radical-trapping antioxidant parameter (TRAP) were measured. LDL susceptibility to oxidation was evaluated at baseline and after 120 minutes. Plasma glucose, insulin, triglycerides, and MDA increased and NEFAs and TRAP significantly decreased after either meal. The variations in plasma glucose, MDA, and TRAP were significantly greater and LDL was more susceptible to oxidation after the meal that produced a significantly higher degree of hyperglycemia. These results suggest that postprandial hyperglycemia may contribute to oxidative stress in diabetic patients, providing a mechanistic link between hyperglycemia and diabetic vascular disease.

THE INCREASED INCIDENCE of cardiovascular disease in patients with type 2 diabetes mellitus cannot be fully explained by elevated levels of the classic risk factors.[1] In recent years, there is growing evidence that oxidative stress, ie, the imbalance between free-radical production and antioxidant defenses, is involved in the pathogenesis of cardiovascular disease in diabetes mellitus.[2-3] The respective roles of hyperglycemia,[4] hyperlipidemia,[5] and, in type 2 diabetes, hyperinsulinemia[6] in atherogenesis and oxidative stress are still a matter of debate.

With regard to the manner in which free radicals may cause vascular damage, their contribution to low-density lipoprotein (LDL) oxidation appears to play a role, since oxidized LDL appears to be more atherogenic than ordinary LDL.[7] According to recent studies in normal subjects, meals are followed by oxidative stress,[8,9] with consumption of antioxidant defenses,[8] increased susceptibility of LDL to oxidation,[10] and endothelial dysfunction.[11] The postprandial generation of an oxidative stress with consumption of antioxidants has also been demonstrated in type 2 diabetic patients,[8] and a higher susceptibility of LDL to oxidation has been reported in type 2[12] and type 1[13] diabetic patients. The evidence that LDLs are more prone to oxidation in poorly controlled[13] versus well-controlled[14] type 1 diabetic patients suggests that LDL oxidation may be conditioned by glycometabolic control.

The life of a diabetic patient is marked by large and rapid increases in the blood level of glucose and lipids (mostly triglycerides), particularly after meals. The possibility that acute hyperglycemia itself can produce an oxidative stress in normal and diabetic subjects has been reported.[15,16] However, in type 2 diabetic patients, the postprandial phase is also associated with hyperinsulinemia. In the postabsorptive phase, phenomena related to atherogenesis are amplified and can be more easily studied. Possibly, this is also the condition in which the atherogenic process is boosted.

To test the relative impact of the blood glucose level on oxidative stress and related LDL modifications in diabetes, we evaluated the effect of two different standard meals, designed to produce different levels of postprandial hyperglycemia, on the plasma oxidative status and the susceptibility of LDL to oxidation in type 2 diabetic patients. Postprandial oxidative balance was assessed by measuring plasma malondialdehyde (MDA), plasma total radical-trapping antioxidant parameter (TRAP), and LDL susceptibility to oxidation. MDA is a widely used index of lipid peroxidation,[5] whereas TRAP is a global measure of the antioxidant capacity of plasma, taking into account all known and unknown antioxidant activity present in plasma, as well as their synergy.[17] In fact, the overall antioxidant capacity of plasma is determined not only by the absolute concentration of the various antioxidant compounds but also by their interactions.[18]

SUBJECTS AND METHODS

Subjects

Informed consent to participate in the study was obtained from 10 type 2 diabetic patients (six men and four women; age, 54.1 ± 1.5 years (mean ± SE); duration of diabetes, 7.0 ± 1.2 years; body mass index, 25.6 ± 1.1 kg/m^2) after a clear explanation of its experimental nature. The study protocol was approved by the Ethics Committee of our institution.

The subjects had a hemoglobin A_{1c} (HbA_{1c}) level of 7.1% ± 0.3%, (range, 6.4% to 8.1%) and were under treatment with a standard diet for diabetic patients (50% carbohydrate, 20% protein, and 30% fat) and the hypoglycemic agent glybenclamide (daily dose, 2.5 to 7.5 mg). Dietary information was obtained by trained interviewers using a previously validated questionnaire.[19] All subjects were nonsmokers.

From the Department of Pathology and Medicine, Clinical and Experimental, University of Udine, Udine; Diabetology Unit, Udine General Hospital, Udine; and Department of Geriatric Research, Cytology Unit, Istituto Nazionale Riposa e Cura Anziani, Ancona, Italy.

Submitted September 21, 1998; accepted June 10, 1999.

Address reprint requests to Antonio Ceriello, MD, Chair of Internal Medicine, University of Udine, P.le S. Maria della Misericordia, 33100 Udine, Italy.

0026-0495/99/4812-0010$10.00/0

None of the subjects were on antioxidant supplementation. None had macroangiopathy as judged by pathological changes on a resting electrocardiogram or a history of cardiac angina, myocardial or cerebral infarction, or intermittent claudication. None had microalbuminuria, macroalbuminuria, or retinopathy.

Study Design

We designed a randomized crossover study in which each subject received, in random order, two meals (A and B; Table 1) differing mainly in carbohydrate content. All medications were withheld from 3 days before to the end of the study, and the patients maintained their standard diet throughout the study.

The two types of meals were (A) Ensure Plus (400 mL: carbohydrate 80 g, lipid 20 g, and protein 25 g) and (B) Glucerna (500 mL: carbohydrate 47 g, lipid 28 g, and protein 21 g). Formula foods were chosen in order to achieve a high level of reproducibility, which could not be guaranteed by hand-made meals. The two formulations were balanced for vitamins and other trace elements, and the different carbohydrate contents were supposed to produce a different postprandial hyperglycemic response (Table 1). Smaller differences in fat and protein content could not be avoided with these formula foods, but we believe that their influence on the results would not interfere, at least in our acute tests, with the dominant influence of the carbohydrate component. Lipids were derived from vegetable oil in accordance with American Diabetes Association recommendations[20] (safflower oil 85% enriched with oleic acid and soybean oil 15% in Glucerna, and corn oil 15% in Ensure).

Between 9 and 11 AM after a 12- to 14-hour fast, each subject consumed, in 10 minutes, one of the two types of meals. Blood samples obtained in the absence of venous stasis were drawn at baseline and 60 and 120 minutes after the meal. In every sample, plasma levels of glucose, insulin, cholesterol, triglyceride, nonesterified fatty acid (NEFA), MDA, and TRAP were measured. LDL susceptibility to oxidation was evaluated at baseline and after 120 minutes. The procedure was repeated 7 days later with the other type of meal.

Table 1. Meal Composition

Component	Meal A	Meal B
Water (g)	308	437
Protein (g)	25	21
Fat (g)	20	28
Carbohydrate (g)	80	47
Calcium (mg)	424	422
Phosphorus (mg)	424	422
Magnesium (mg)	168	153
Iron (mg)	7.6	7.5
Zinc (mg)	9.6	9.3
Manganese (mg)	2.32	2.1
Copper (mg)	0.8	0.8
Iodine (pg)	16	86.22
Sodium (mg)	472	557.9
Potassium (mg)	728	938.4
Chlorine (mg)	644	862.5
Chromium (pg)	40	51.6
Vitamin A (pg)	634	678
Vitamin D (pg)	4.2	4.1
Vitamin E (mg)	14	15
Vitamin K (pg)	30	38
Vitamin B_1 (mg)	1.2	1.5
Vitamin B_2 (mg)	1.4	1.5
Vitamin B_6 (mg)	1.7	1.9
Vitamin B_{12} (pg)	5.2	6.3
Niacin (mg)	16.8	15.4
Folic acid (pg)	340	295
Pantothenic acid (mg)	8.4	8.4
Biotin (pg)	252	259
Vitamin C (mg)	100	102
Choline (mg)	220	323
Selenium (pg)	34	28
Molybdenum (pg)	72	68

Assays

Plasma glucose was determined by the glucose-oxidase method and insulin by a radioimmunologic method. The HbA_{1c} level was measured by aminophenylboronic acid affinity chromatography.[21] Cholesterol and triglyceride concentrations were measured enzymatically on a clinical chemistry analyzer (Monarch; Instrumentation Laboratory, Lexington, MA). NEFAs were determined by a commercially available kit (NEFA Quick; Boehringer Mannheim, Tokyo, Japan). MDA was evaluated by a highly sensitive fluorometric method according to Conti et al.[22] The intraassay and interassay coefficients of variation for this method were 6.5% and 8.8%, respectively.

Measurement of TRAP

2,2′-Azobis-(2-amidinopropane) dihydrochloride c(ABAP), R-phycoerythrin (R-PE), Trolox, and all other chemicals were purchased from Sigma Chemical (St Louis, MO). TRAP was evaluated according to Ghiselli et al.[17] In this method, the production of peroxyl radicals obtained by thermal decomposition of ABAP leads to a linear decrease in R-PE fluorescence emission over 1 hour. When plasma is added to the reaction mixture, a period of complete protection of R-PE is observed. The length of this lag-phase (T) is considered to be directly related to total plasma antioxidant capacity. To quantify TRAP, the T produced by plasma is compared with the T produced by a known amount of Trolox. Values for T are calculated by extrapolating the slope of maximal R-PE decay to intersect with the slope of plasma and Trolox protection. The projection of these intersection points on the x-axis yields the T values, which represent the time required to achieve the maximal R-PE peroxidation rates. By comparing the T of plasma with the T of Trolox, taking into account the concentration of Trolox (Conc), the TRAP value of a plasma sample is obtained according to the proportion, Conc Trolox:T Trolox = X:T plasma.[17] The resulting value for X is then multiplied by 2.0 (the stoichiometric factor of Trolox) and by the dilution factor of plasma (250). Values are expressed as micromolars.

Sample preparation. According to the procedure of Ghiselli et al,[17] the reaction mixture consisted of 1.5×10^{-8} mol/L R-PE in 75 mmol/L phosphate buffer, pH 7. Eight microliters of plasma or 30 μL 120-μmol/L Trolox were added to 2.0 mL final vol, and the resulting solution was maintained at 37°C for 5 minutes in 10-mm quartz fluorometer cells. The oxidation reaction was started by adding ABAP to a final concentration of 4.0 mmol/L, and the decay of R-PE fluorescence was monitored every 5 minutes on a Perkin-Elmer (Norwalk, CT) LS-50 Luminescence Spectrometer equipped with a thermostatically controlled cell-holder; monochromators were operating at excitation wavelength 495 nm/5-nm slit width and emission wavelength 575 nm/5-nm slit width. TRAP values were calculated as already described. Intraassay and interassay coefficients of variation for this method were 10% and 12%, respectively. Glucose interference with the TRAP assay has been previously excluded.[9]

Susceptibility of LDL to Oxidation

LDL isolation. Blood samples were obtained by venipuncture and collected into Vacutainer tubes with EDTA as an anticoagulant (Becton Dickinson, Rutherford, NJ). After centrifugation (10 minutes at $2,200 \times g$) at room temperature, LDL was isolated from plasma by the short-run ultracentrifugation procedure described by Kleinveld et al.[23]

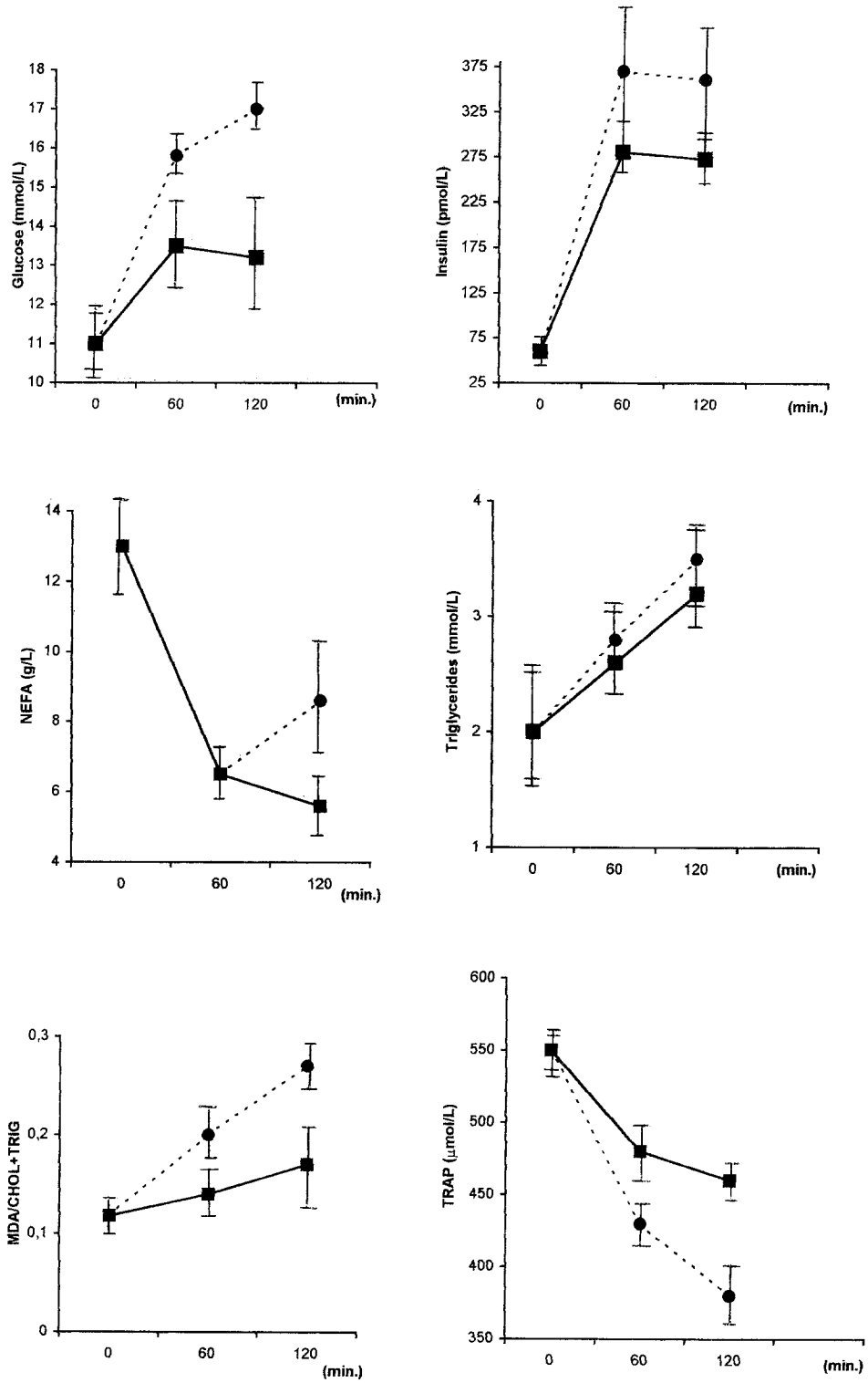

Fig 1. Plasma glucose, insulin, TRAP, triglycerides, NEFA, and MDA at the 0-, 60-, and 120-minute intervals (mean ± SE) after high-carbohydrate (meal A, ●) or low-carbohydrate (meal B, ■) meals in diabetic subjects. ANOVA showed that plasma glucose, insulin, triglycerides, and MDA (corrected for triglyceride + cholesterol concentration [CHOL + TRIG]) significantly increased ($P = .001$), while NEFA and plasma TRAP significantly decreased ($P = .001$) with time in either type of meal test. The variations in plasma glucose, MDA, and plasma TRAP were greater after meals producing more pronounced hyperglycemia, while insulin, NEFA, and triglyceride changes were not significantly different between the 2 meals.

The LDL was separated from EDTA and from diffusible low–molecular-weight species by gel filtration on AcA22 gel (IBF, Creteil, France). The column (1.6 × 20 cm) was previously equilibrated in 10 mmol/L phosphate-buffered saline, pH 7.4, bubbled continuously with argon to flush dissolved oxygen. The fractions eluting at the void volume were pooled. The protein concentration was determined by the method of Lowry et al[24] and the LDL was diluted with phosphate-buffered saline to a protein concentration of 0.05 mg/mL, filtered through a 0.45-µm (pore size) filter, and used immediately.

LDL oxidation. Oxidation was initiated by adding a freshly prepared $CuSO_4$ solution (final concentration, 5 µmol/L). The kinetics of LDL oxidation was determined by monitoring the change in absorbance

of the conjugated dienes at 234 nm at 37°C with a Beckman (Fullerton, CA) DU-7 spectrophotometer. Absorbance was recorded every 5 minutes for about 5 hours. From the change in absorbance at 234 nm versus time, three parameters were determined: the duration of the lag phase time of diene peak, and rate of diene formation.[25]

Statistical Analysis

Statistical analysis of the data was performed using the BMDP Statistical Software package (BMDP Statistical Software, Berkeley, CA). ANOVA for repeated measures was used to identify significant differences in the mean value for every variable at different times of the specified experiment and between the two experiments. To evaluate the difference in LDL susceptibility to oxidation, a matched *t* test was used. All values are expressed as the mean ± SE.

RESULTS

Values for the variables after the standard meals A and B are illustrated in Fig 1. Plasma glucose ($F = 18.5$, $P = .001$), insulin ($F = 29.8$, $P = .001$), triglyceride ($F = 12.1$, $P = .001$), and MDA (corrected for triglyceride plus cholesterol concentration[26]; $F = 26.2$, $P = .001$) significantly increased and NEFA ($F = 10.2$, $P = .001$) and TRAP ($F = 80.8$, $P = .001$) significantly decreased with time for either type of meal test. The increase in plasma glucose ($F = 28.4$, $P = .001$) and MDA ($F = 36.9$, $P = .001$) and the decrease in plasma TRAP ($F = 33.2$, $P = .001$) were significantly greater after meal A versus meal B. Variations in plasma triglyceride levels were similar for the two types of meal tests, and plasma insulin concentrations were higher, but not to a significant degree, after meal A. NEFA levels increased at 120 minutes compared with the 60-minute interval after meal A, but the ANOVA, taking into account the variation throughout the experiment, did not point out this datum.

LDL susceptibility to oxidation in terms of the duration of the lag phase ($P < .01$), time of diene peak ($P < .01$), and rate of diene formation ($P < .01$) significantly increased at 2 hours after the meal versus baseline in both experimental conditions. Two hours after the meals, LDL was more susceptible to oxidation with the meal (A) that produced a higher degree of hyperglycemia ($P < .05$) (Fig 2).

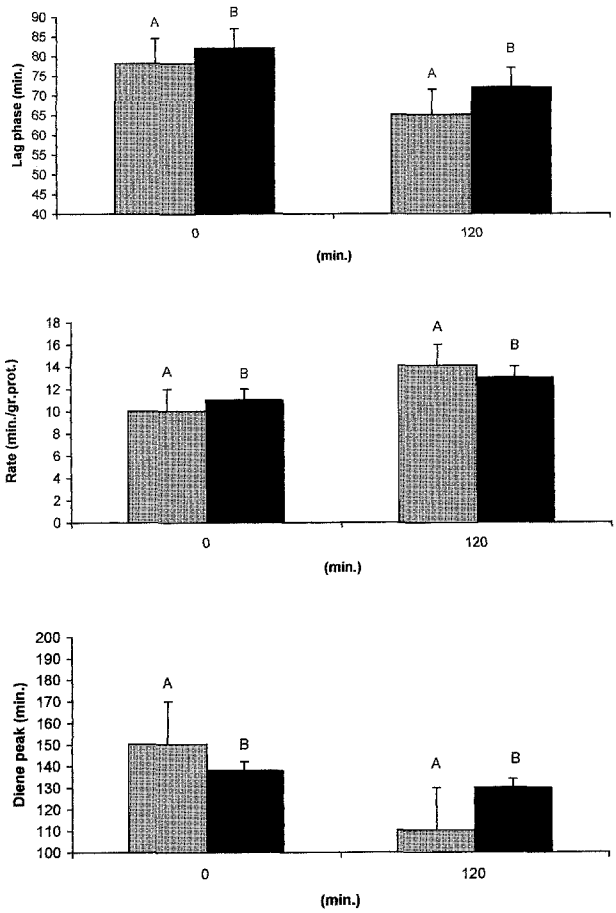

Fig 2. LDL oxidation at baseline and 2 hours after high-carbohydrate (A) or low-carbohydrate (B) meals in diabetic subjects. LDL susceptibility to oxidation in terms of the lag phase duration ($P < .01$), time of diene peak ($P < .01$), and rate of diene formation ($P < .01$) significantly increased at 2 hours after the meal *v* baseline in both experimental conditions. LDL was more susceptible to oxidation after type A meals ($P < .05$).

DISCUSSION

Oxidative stress has been proposed as a pathogenetic factor of atherosclerosis in diabetes.[2] In recent years, there is growing evidence that oxidative modifications of LDL greatly increase its atherogenicity.[7] The susceptibility of LDL to oxidative modification has been reported to be increased in diabetic patients,[12,13] and it has been suggested that a high glucose concentration[26,27] and/or reduction of plasma antioxidant defenses[13] may contribute to this phenomenon.

Our study confirms that meals are followed by a substantial decrease in plasma TRAP and an increase in MDA in type 2 diabetic patients.[8] Moreover, in our diabetic patients, the susceptibility of LDL to oxidation significantly increases after meals. These effects are more pronounced after a type of meal that produces a larger increase in plasma glucose. These results are consistent with the in vitro evidence that high concentrations of glucose promote oxidative modification of LDL,[27] possibly through a free-radical pathway,[28] and with the in vivo finding that LDL oxidation is higher in poorly controlled diabetic patients.[13] In accordance with Tsai et al,[13] we hypothesize that in our study the postprandial reduction of antioxidant defenses, as indicated by the TRAP decrease, may significantly contribute to increased LDL oxidizability.

During an acute increase of blood glucose, the presence of labile nonenzymatic glycation (Schiff base formation) and of an intracellular imbalance of the NADH/NAD^+ ratio, processes that have been shown to occur very rapidly, may provoke oxidative stress, since it has been shown that both of these phenomena are accompanied by the production of free radicals.[29,30] However, the postprandial increase in insulin and triglyceride might also be related to oxidative stress. It has been suggested that insulin, or at least hyperinsulinemia, may directly induce intracellular production of free radicals.[6] Conversely, insulin resistance may be increased by oxidative stress.[31] In our study, there was a trend for a higher insulin response to the type A meal compared with the type B meal, although this difference was not statistically significant. Possi-

bly, the relatively small number of subjects did not permit a real difference to emerge.

Another difference that did not reach the threshold of statistical significance in ANOVA is the certain discrepancy in NEFA levels at 120 minutes after the two types of meals. Since NEFA reduction is induced postprandially by the insulin surge, partially counterbalanced by the increase in blood glucose,[32] the different levels of glucose and insulin after meal A versus meal B may have interacted to produce the reported variation in NEFA.

Triglycerides increased in a similar measure after either type of meal. Plasma triglycerides in the postabsorptive state derive partly from absorbed lipids and partly from glucose-induced hepatic synthesis.[33] The two types of meals we used contained 20 and 28 g lipid, respectively, mostly represented by triglycerides given the vegetable oil source. Probably after meal A, a larger hyperglycemia-dependent endogenous production compensated for the relatively smaller exogenous triglyceride quantity. The net result was a triglyceride variation that was similar after the two types of meals. Consequently, although they were possibly responsible for part of the oxidative stress,[5] triglycerides did not contribute to the differences between the two meal tests.

In conclusion, our study demonstrates that in diabetic patients, meals are associated with the generation of oxidative stress that produces antioxidant consumption. This phenomenon may consistently contribute to increase LDL susceptibility to oxidation. The role of glucose appears prominent, since TRAP reduction and the susceptibility of LDL to oxidation are greater after meals that cause higher plasma glucose. Several epidemiological investigations have demonstrated that a poorly controlled blood glucose concentration in diabetic patients significantly contributes to the development of macroangiopathic complications,[34] while in animal models a direct role of hyperglycemia in favoring atherosclerosis has been evidenced.[35] Moreover, the possibility has been recently proposed that postprandial "hyperglycemic spikes" may play a direct role in the pathogenesis of late diabetic complications.[36] From a clinical standpoint, the message should be to increase efforts to control postprandial hyperglycemia in diabetic patients to better prevent the development of atherosclerosis.

REFERENCES

1. Kannel WB, McGee DL: Diabetes and cardiovascular risk factors: The Framingham Study. Circulation 59:8-13, 1979
2. Giugliano D, Ceriello A, Paolisso G: Oxidative stress and diabetic vascular complications. Diabetes Care 19:257-267, 1996
3. Baynes JW: Role of oxidative stress in development of complications in diabetes. Diabetes 40:405-412, 1991
4. Ceriello A, Giugliano D: Oxidative stress and diabetic complications, in Alberti KGMM, Zimmet P, De Fronzo RA (eds): International Textbook of Diabetes Mellitus (ed 2). Chichester, UK, Wiley, 1997, pp 1453-1461
5. Janero DR: Malondialdehyde and thiobarbituric acid–reactivity as diagnostic indices of lipid peroxidation and peroxidative tissue injury. Free Radic Biol Med 9:515-540, 1990
6. Ceriello A, Pirisi M: Is oxidative stress the missing link between insulin resistance and atherosclerosis? Diabetologia 38:1484-1485, 1995 (letter)
7. Witzum JL: Susceptibility of low-density lipoprotein to oxidative modification. Am J Med 94:347-349, 1993
8. Ceriello A, Bortolotti N, Motz E, et al: Meal-generated oxidative stress in type 2 diabetic patients. Diabetes Care 21:1529-1533, 1998
9. Ursini F, Zamburlini A, Cazzolato G, et al: Postprandial plasma lipid hydroperoxides: A possible link between diet and atherosclerosis. Free Radic Biol Med 25:250-252, 1998
10. Lechleitner M, Hoppichler F, Föger B, et al: Low-density lipoproteins of the postprandial state induce cellular cholesteryl ester accumulation in macrophages. Arterioscler Thromb 14:1799-1807, 1994
11. Plotnick GD, Corretti MC, Vogel RA: Effect of antioxidant vitamins on the transient impairment of endothelium-dependent brachial artery vasoactivity following a single high-fat meal. JAMA 278:1682-1686, 1997
12. Babiy AV, Gebicki JM, Sullivan DR, et al: Increased oxidizability of plasma lipoproteins in diabetic patients can be decreased by probucol therapy and is not due to glycation. Biochem Pharmacol 43:995-1000, 1992
13. Tsai EC, Hirsch IB, Brunzell JD, et al: Reduced plasma peroxyl radical trapping capacity and increased susceptibility of LDL to oxidation in poorly controlled IDDM. Diabetes 43:1010-1014, 1994
14. Jenkins AJ, Klein RL, Chassereau CN, et al: LDL from patients with well-controlled IDDM is not more susceptible to in vitro oxidation. Diabetes 45:762-767, 1996
15. Ceriello A: Acute hyperglycaemia and oxidative stress generation. Diabet Med 14:S45-S49, 1997 (suppl)
16. Ceriello A, Bortolotti N, Crescentini A, et al: Antioxidant defenses are reduced during oral glucose tolerance test in normal and non–insulin dependent diabetic subjects. Eur J Clin Invest 28:329-333, 1998
17. Ghiselli A, Serafini M, Maiani G, et al: A fluorescence-based method for measuring total plasma antioxidant capability. Free Radic Biol Med 18:29-36, 1995
18. Halliwell B, Gutteridge JMC: The antioxidants of human extracellular fluids. Arch Biochem Biophys 280:1-8, 1990
19. Block G, Hartman AM, Dresser CM, et al: A data-based approach to diet questionnaire design and testing. Am J Epidemiol 124:453-469, 1986
20. American Diabetes Association: Nutrition recommendations and principles for people with diabetes mellitus. Diabetes Care 21:S32-S35, 1998 (suppl 1)
21. Mallia AK, Heremenson GT, Krohn RS, et al: Preparation and use of a boronic acid affinity chromatography for separation and quantification of glycosylated hemoglobin. Anal Lett 14:649-661, 1981
22. Conti M, Morand PC, Levillain P, et al: Improved fluorometric determination of malonaldehyde. Clin Chem 37:1273-1275, 1991
23. Kleinveld HA, Hak-Lemmers HLM, Stalenhoef AFH, et al: Improved measurement of low-density lipoprotein susceptibility to copper-induced oxidation: Application of a short procedure for isolating low-density lipoprotein. Clin Chem 38:2066-2072, 1992
24. Lowry OH, Rosebrough NJ, Farr AL, et al: Protein measurement with the Folin phenol reagent. J Biol Chem 193:265-275, 1951
25. Esterbauer H, Striegl G, Puhl H, et al: Continuous monitoring of in vitro oxidation of human low density lipoprotein. Free Radic Res Commun 6:67-75, 1989
26. Nacitarhan S, Ozben T, Tuncer N: Serum and urine malondialdehyde levels in NIDDM patients with and without hyperlipidemia. Free Radic Biol Med 19:893-896, 1995
27. Hunt JV, Smith CC, Wolff SP: Autoxidative glycosylation and

possible involvement of peroxides and free radicals in LDL modification by glucose. Diabetes 39:1420-1424, 1990

28. Kawamura M, Heinecke JW, Chait A: Pathophysiological concentrations of glucose promote oxidative modification of low density lipoprotein by a superoxide-dependent pathway. J Clin Invest 94:771-778, 1994

29. Mullarkey CJ, Edelstein D, Brownlee M: Free radical generation by early glycation products: A mechanism for accelerated atherogenesis in diabetes. Biochem Biophys Res Commun 173:932-939, 1990

30. Hubinont C, Sener A, Malaisse WY: Sorbitol content of plasma and erythrocytes during short-term hyperglycemia. Clin Biochem 14:19-20, 1981

31. Paolisso G, Giugliano D: Oxidative stress and insulin action: Is there a relationship? Diabetologia 39:357-363, 1996

32. Fraze E, Donner CC, Swislocki ALM, et al: Ambient plasma free fatty acid concentrations in non–insulin dependent diabetes mellitus: Evidence for insulin resistance. J Clin Endocrinol Metab 61:807-811, 1985

33. Howard BV: Lipoprotein metabolism in diabetes mellitus. J Lipid Res 28:613-628, 1987

34. Laakso M: Hyperglycemia and cardiovascular disease in type 2 diabetes. Diabetes 48:937-942, 1999

35. Kunjathoor VV, Wilson DL, LeBoeuf RC: Increased atherosclerosis in streptozotocin-induced diabetic mice. J Clin Invest 97:1767-1774, 1996

36. Ceriello A: The emerging role of post-prandial hyperglycaemic spikes in the pathogenesis of diabetic complications. Diabet Med 15:188-193, 1998