Free Fatty Acid–Mediated Impairment of Glucose-Stimulated Insulin Secretion in Nondiabetic Oji-Cree Individuals From the Sandy Lake Community of Ontario, Canada

A Population at Very High Risk for Developing Type 2 Diabetes

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The Oji-Cree population of the Sandy Lake region of Ontario, Canada, has the third highest prevalence of type 2 diabetes in the world. Changes in their diet and physical activity over the past half-century, particularly the marked increase in consumption of dietary fats, are felt to be important factors accounting for this epidemic. The aim of the present study was to examine the β-cell response to a 48-h approximately twofold elevation of plasma free fatty acids (FFAs) (induced by Intralipid and heparin infusion) in members of the Sandy Lake Oji-Cree population (n = 12) and to compare the response to that in healthy age-matched nondiabetic Caucasian subjects (n = 16). The insulin secretion rate, insulin sensitivity index (S_I), and disposition index (D_I) (an index of insulin secretion that takes into account the ambient S_I) were assessed in response to a 4-h graded intravenous glucose infusion followed by a 20 mmol/l 2-h hyperglycemic clamp. Total insulin secretory response to the graded glucose infusion did not change after a 48-h FFA elevation versus saline control in Caucasians and increased by ~30% in Oji-Cree individuals (P = 0.04 for difference between the two groups). Infusion of heparin-Intralipid reduced S_I by ~40% in both groups (P = 0.002). Although D_I was markedly reduced by heparin-Intralipid infusion in Caucasians (by ~40%), it was reduced by only 15% in Oji-Cree individuals (P = 0.03 for difference of response between the two groups). However, S_I and D_I in the Oji-Cree individuals were already much lower than in Caucasians at baseline, in keeping with the very high risk of type 2 diabetes in this population. It is concluded that Oji-Cree individuals from a community at very high risk for developing type 2 diabetes are not more susceptible to the FFA-induced desensitization of glucose-stimulated insulin secretion than healthy non-Natives and, in fact, appear to be less susceptible. Whether this reflects an inherent resistance to lipotoxicity or an already-present lipotoxic effect in this population will require further study. *Diabetes* 52:1485–1495, 2003

Type 2 diabetes is characterized by defects in both insulin action and insulin secretion (1,2), with a specific defect in glucose-stimulated insulin secretion (GSIS) early in the evolution of this disease (3,4). Prolonged elevation of plasma free fatty acids (FFAs) has repeatedly been shown to desensitize pancreatic β-cell GSIS in ex vivo and in vitro studies (5–9). The concept of FFA-induced β-cell desensitization has received support from epidemiological prospective studies, showing that elevated plasma FFA levels are a risk marker for the long-term development of glucose intolerance and progression toward type 2 diabetes, both in Caucasians (10) and in Pima Indians (11). In addition, experimental evidence in animals supports a role for prolonged elevation of plasma FFAs in the desensitization of GSIS (12,13). Some investigators, including our group, have shown evidence for a lipotoxic effect on β-cell function also in humans (14–17), whereas others have not (18).

Genetic factors are clearly involved in the pathogenesis of β-cell dysfunction because some ethnic groups are clearly at higher risk of developing type 2 diabetes, independent of other risk factors (19,20). Recently, a private mutation of the *HNF1α* gene (MIM 142410.0008), G319S, has been associated with a very high risk of developing type 2 diabetes in the Oji-Cree population of the Sandy Lake region in the northwest part of Ontario, Canada (21,22). Nevertheless, the Oji-Cree population from the Sandy Lake region who do not have this mutation also display a high risk of developing type 2 diabetes compared

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D_I, disposition index; FFA, free fatty acid; G_{inf}, glucose infusion rate; GSIS, glucose-stimulated insulin secretion; HI, heparin-Intralipid; ISR, insulin secretion rate; S_I, insulin sensitivity index.

with Canadian populations of Caucasian descent (22). A strong case can be made in the Oji-Cree population for a potential lipotoxic effect on the pancreatic β-cell, because it has been clearly demonstrated in this population that a high consumption of fatty foods and more fatty methods of food preparation are associated with an increased risk for diabetes (23). Although the ingestion of a high-fat diet and reduction in physical activity may have precipitated β-cell failure by inducing obesity and insulin resistance, there may also be a direct effect of elevated FFAs on β-cell function.

We undertook this study to test the hypothesis that young and healthy Oji-Cree individuals with normal glucose tolerance could be at higher risk of developing FFA-induced desensitization of GSIS than healthy Caucasian individuals, perhaps making them more susceptible to the diabetogenic effect of a high-fat diet.

RESEARCH DESIGN AND METHODS

Subjects. Twelve Oji-Cree individuals from the Sandy Lake region (nine men and three women) participated in the study. Table 1 shows their demographic and clinical characteristics. These participants traveled by commercial airline to Toronto 3 days before the study and were admitted to the Metabolic Investigation Unit of the Toronto General Hospital to undergo the metabolic studies described below. A standard 2-h oral glucose tolerance test was performed in all these subjects to exclude the presence of impaired glucose tolerance or diabetes (24). All of the participants were healthy as assessed by medical history; physical examination; liver, kidney, and thyroid function tests; and plasma lipid profile. None were taking any medication. The three women who participated were premenopausal and had a normal menstrual cycle. Data from the Oji-Cree individuals were compared with data from 16 healthy Caucasian males with similar age and BMI, previously studied in our laboratory using similar research protocols (Table 1) (16). Informed written consent was obtained from all participants in accordance with the guidelines of the Human Subjects Review Committee of The Toronto Hospital, University of Toronto, and the study was approved by the Sandy Lake First Nation band council.

Experimental protocols. For practical considerations, Sandy Lake subjects flying a long distance to Toronto to participate in these physiological studies underwent an oral glucose tolerance test, followed by a saline control study of pancreatic function and then a 48-h infusion of Intralipid and heparin followed by testing of pancreatic function, performed sequentially during a single 5-day hospital admission (Fig. 1). For the Caucasian control subjects, the protocol used to test pancreatic function was identical, but the saline control study was performed 4–6 weeks apart from the 48-h Intralipid and heparin infusion study, on separate admissions to the hospital, as previously described (16). The weight of the Caucasian participants did not change between the two studies (76.3 ± 2.3 vs. 76.2 ± 2.5 kg in the heparin-Intralipid [HI] study vs. the saline study, respectively). The protocol for the Sandy Lake subjects was as follows: after a 12-h overnight fast, an intravenous catheter was placed in each forearm (one for infusion and one for blood sampling) and the participants underwent a standard 75-g oral glucose tolerance test on day 1. Thereafter, they received an isocaloric diet containing 50% carbohydrates, 30% fat, and 20% proteins for the duration of their 5-day stay in the hospital. After a 24-h intravenous infusion of saline and an overnight fast, on day 2, they underwent a graded intravenous glucose infusion study followed by a 2-h 20 mmol/l hyperglycemic clamp, as previously described (16). This protocol was designed to measure both the insulin secretory response to an incremental rise in blood glucose as well as a constant near-maximally stimulated insulin secretory response to marked constant hyperglycemia. At ~8:00 A.M. on day 2, with the intravenous saline infusion continuing at a steady rate, a graded intravenous infusion of glucose (20% dextrose) was then started at a rate of 1 $mg \cdot kg^{-1} \cdot min^{-1}$, followed by infusions of 2, 3, 4, 6, and 8 $mg \cdot kg^{-1} \cdot min^{-1}$ for a period of 40 min at each glucose infusion rate (G_{inf}). Samples were drawn for measurement of glucose, insulin, C-peptide, FFAs, and triglycerides every 10 min at baseline and throughout the experiment. At the end of this period, a 2-h 20 mmol/l hyperglycemic clamp was initiated according to the modified method of DeFronzo et al. (25), and plasma insulin, C-peptide, FFA, and triglyceride levels were measured during the last 30 min of the clamp. This intervention will be referred to as the "saline study" throughout the article.

On day 3, 20% Intralipid (40 ml/h; Baxter, Mississauga, Canada) and heparin sodium (250 units/h; Organon Teknika, Toronto, Canada) infusions were started at 8:00 A.M. and continued for 48 h to raise plasma FFA levels throughout days 3 and 4 of the protocol, for a total of 48 h. On day 5, with the HI infusions continuing, a 4-h graded intravenous glucose infusion study followed by a 2-h 20 mmol/l hyperglycemic clamp was performed after an overnight fast, as describe above. This intervention will be referred to as the "HI study" throughout the article.

***HNF1α* genotyping.** The presence of the G319S allele of the *HNF1α* gene was determined in all of the participants, as previously described (21). Of the 12 participants (25%), 3 were shown to be heterozygous carriers of this allele (Table 1), in accordance with the previously described frequency of this mutation in this population (21).

Laboratory methods. Glucose was assayed enzymatically at the bedside using a Beckman Glucose Analyzer II (Beckman Instruments, Fullerton, CA). Insulin and C-peptide were measured by radioimmunoassay using a double-antibody separation method (kit supplied by Pharmacia Diagnostic, Uppsala, Sweden, and by Diagnostic Products, Los Angeles, CA) as previously described (15). The samples for all studies in the same patient were assayed simultaneously with the same kit for both insulin and C-peptide. FFAs were measured by a colorimetric method (kit supplied by Wako Industrials, Osaka, Japan). Triglycerides were measured as esterified glycerol using an enzymatic colorimetric kit (Boehringer Mannheim Diagnostica).

Calculations

Estimation of the insulin secretion rate. Pancreatic insulin secretion rate (ISR) was calculated from peripheral plasma C-peptide levels by deconvolution using a two-compartment mathematical model with standard parameters for C-peptide distribution and metabolism as previously described (26) (the software program for calculation of insulin secretion was provided by Drs. K. Polonsky and J. Sturis, University of Chicago, Chicago). The use of standard parameters for C-peptide clearance and distribution has been shown to result in ISRs that differ in each subject by only 10–12% from those obtained with individual parameters and there is no systematic over- or underestimation of insulin secretion (26). The C-peptide kinetic parameters are not affected by infusion of heparin and Intralipid (27).

Method of analysis of the relationship between glucose and ISR, insulin, and C-peptide during the graded intravenous glucose infusion studies. Baseline levels of glucose, insulin, ISR, FFAs, and triglycerides were calculated as the mean of the four baseline samples in each study. During the graded glucose infusion protocol, average levels of these parameters were also calculated for the last 20 min of the 40-min period for each infusion rate. Mean ISR, insulin level, and C-peptide level for each period were then plotted against the corresponding mean glucose level, thereby establishing a dose-response relationship between glucose and these variables. To statistically analyze the results, the average ISR, insulin level, and C-peptide level over each sequential 1 mmol/l glucose concentration interval between 6 and 9 mmol/l was calculated in each individual as the area under the curve using the trapezoidal rule. This area was then divided by 1 mmol/l to obtain the correct units (pmol/min for ISR, pmol/l for insulin, and nmol/l for C-peptide).

Insulin sensitivity index and disposition index. The insulin sensitivity index (S_I) was calculated during the 20 mmol/l hyperglycemic clamp studies according to the following (28):

$$S_I = Cl_{glu}/(Ins_{clamp} - Ins_{baseline})$$

Where Cl_{glu} is the glucose clearance during the hyperglycemic clamp, estimated as the G_{inf} divided by the plasma glucose concentration during the last 30 min of the clamp; Ins_{clamp} is the insulin concentration during the last 30 min of the glucose clamp; and $Ins_{baseline}$ is the mean insulin level during the baseline period. S_I is reported in units of dl/kg/min per μU/ml. No correction was made for the urine glucose loss because the urine glucose loss during the hyperglycemic clamp was equal between the saline and HI studies (data not shown). Further, because the plasma insulin levels were >450 pmol/l during the 20 mmol/l hyperglycemic clamp, the endogenous glucose production was assumed to be zero. The disposition index (D_I) was then calculated for each experimental period as an index of correction of ISR for the ambient degree of insulin sensitivity as the product of S_I and ISR (29). D_I is given in arbitrary units, which are defined as liters squared per kilogram per minute (2). In our Oji-Cree population, we were able to demonstrate the assumed hyperbolic relationship between ISR calculated by deconvolution of plasma C-peptide levels and S_I ($R^2 = 0.34$, $P = 0.03$).

Insulin clearance. Clearance of endogenous insulin was calculated by dividing the mean ISR by the mean serum insulin in the last 20 min of each period of glucose infusion of the graded glucose infusion protocol (30). Because ISR and insulin concentrations tended to plateau during this period, the inaccuracy associated with the non–steady state was reduced. Insulin clearance was also calculated during the last 30 min of the 20 mmol/l hyperglycemic clamp.

TABLE 1
Characteristics of the patients

	Age (years)	Sex	BMI (kg/m^2)	Fasting glucose (mmol/l)	2 h after 75-g OGTT	Fasting plasma FFAs (mmol/l)	Fasting plasma insulin (pmol/l)	Fasting plasma C-peptide (mmol/l)	Fasting plasma triglycerides (mmol/l)	Mutation G319S of *HNF1α*
Oji-Cree participants from Sandy Lake (patient number)										
1	26	M	27.0	4.9	5.5	0.302	58.6	0.08	0.54	−/−
2	23	M	24.8	5.1	4.1	0.304	43.0	0.02	0.82	−/−
3	29	M	24.9	5.4	6.3	0.242	68.4	0.04	1.22	−/−
4	27	M	32.4	5.4	5.6	0.258	56.0	0.09	0.76	−/−
5	20	M	25.5	5.5	6.6	0.456	52.8	0.47	1.58	−/−
6	24	M	24.0	5.1	4.1	0.283	34.6	0.35	1.18	−/−
7	32	F	31.3	5.3	5.8	0.562	53.8	0.22	0.39	−/−
8	20	F	31.6	5.4	5.2	0.405	98.4	0.54	1.10	−/−
9	25	M	22.0	5.0	6.4	0.398	45.4	0.32	1.26	+/−
10	21	F	28.0	4.8	4.5	0.684	57.6	0.39	0.55	−/−
11	24	M	23.7	5.2	4.0	0.723	43.4	0.44	0.74	+/−
12	22	M	19.9	4.5	4.4	0.375	29.4	0.38	0.58	+/−
Oji-Cree group	24 ± 1	—	26.3 ± 1.1	5.1 ± 0.1	5.2 ± 0.3	0.416 ± 0.047	53.5 ± 5.2	0.28 ± 0.05	0.89 ± 0.11	—
		All								
Caucasians* (n = 16)	25 ± 1	M	24.2 ± 0.5	5.2 ± 0.1	—	0.537 ± 0.046	35.2 ± 2.9	0.34 ± 0.04	0.96 ± 0.08	—
P†	0.59	—	0.09	0.74	—	0.08	0.003	0.38	0.60	—

Data for Oji-Cree group and Caucasians are means ± SE. OGTT, oral glucose tolerance test. *Previously studied Caucasian subjects (16). †P value of unpaired two-tailed t test of Oji-Cree vs. Caucasian participants.

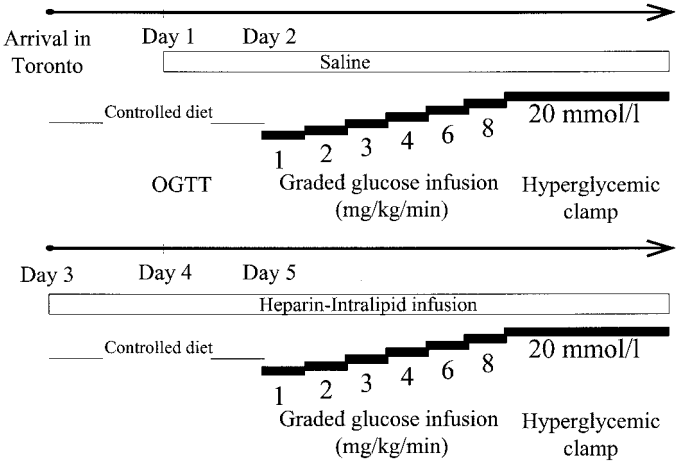

FIG. 1. Five-day experimental protocol for Oji-Cree study participants. See text for details.

Statistical analysis. The data were expressed as means ± SE. Baseline characteristics were compared by an unpaired *t* test between the Oji-Cree individuals and the Caucasian group previously studied. ANOVA for repeated measures that included the experimental protocol (saline vs. HI), ethnicity (Oji-Cree vs. Caucasians), glucose infusion step or glucose area intervals, and interaction term between experimental protocol and ethnicity was performed to analyze the plasma glucose, insulin, C-peptide, ISR, FFA, triglyceride, and insulin clearance response during the graded intravenous glucose infusion studies. ANOVA for repeated measures that included the experimental protocol (saline vs. HI), ethnicity (Oji-Cree vs. Caucasians), and interaction between these factors was performed to analyze the response during the 20 mmol/l hyperglycemic clamp studies. When the interaction term of experimental protocol and ethnicity had a *P* value <0.05, the change in percentage induced by 48-h intravenous infusion of heparin and Intralipid over the saline experiment was compared between the two ethnic groups by ANOVA to assess the difference of response between the two ethnic groups. To detect any confounding effect of the slight difference in body adiposity between the two ethnic groups, all of the analyses were performed after adjustment for BMI using ANCOVA. This did not change our major conclusion and, therefore, we are only reporting the *P* values from the analyses after adjustment for BMI. All of the analyses were performed with and without inclusion of the patients heterozygous for the G319S mutation of the *HNF1α* gene to determine whether inclusion of these individuals altered the results. Calculations were performed with SAS software (SAS Statistical Analysis System, version 8.02; SAS, Cary, NC).

RESULTS

Fasting plasma glucose, insulin, C-peptide, FFA, and triglyceride concentrations in the saline versus 48-h HI study. Fasting plasma glucose, FFAs, triglycerides, and C-peptide were similar during the saline infusion (Table 1) and after the 48-h HI infusion (Table 2) in Oji-Cree and Caucasian subjects. However, fasting plasma insulin levels were significantly higher in the former during the saline study ($P = 0.003$) as well as after a 48-h elevation of plasma FFAs ($P = 0.01$). Infusion of HI for 48 h resulted in a significant elevation of fasting plasma glucose, FFA, triglyceride, insulin, and C-peptide levels in both the Oji-Cree and the Caucasian group (Table 2). However, the elevation of fasting plasma C-peptide levels during HI infusion was significantly higher in Oji-Cree individuals than in Caucasian subjects (141 ± 34% vs. 37 ± 16%, respectively; $P = 0.005$). Exclusion of the three heterozygous carriers of the G319S mutation of the *HNF1α* gene did not change this result.

Graded glucose infusion studies

Plasma glucose, FFAs, and triglycerides versus G_{inf}. Plasma glucose levels (Fig. 2*A*) were slightly but significantly higher after the 48-h HI infusion versus saline ($P < 0.0001$) throughout the graded glucose infusion period and were similar in both ethnic groups. There was an approximately twofold elevation of plasma FFA (Fig. 2*B*) and triglyceride (Fig. 2*C*) levels after the 48-h HI versus saline infusion in both groups ($P < 0.0001$), but the FFA and triglyceride levels in both experimental protocols were slightly lower in the Oji-Cree group than in the Caucasians, even when adjusted for BMI ($P = 0.03$ and $P = 0.02$, respectively). However, excluding the three subjects with the G319S mutation eliminated these differences between both ethnic groups.

TABLE 2
Fasting plasma glucose, insulin, C-peptide, FFA, and triglyceride levels after intravenous infusion of heparin and Intralipid for 48 h

	G319S HNF1α alleles	Glucose (mmol/l)	Insulin (pmol/l)	C-peptide (nmol/l)	Fasting ISR (pmol/min)	FFAs (mmol/l)	Triglycerides (mmol/l)
Oji-cree							
48-h HI		5.6 ± 0.1	93 ± 10	0.49 ± 0.10	135 ± 29	0.901 ± 0.079	1.86 ± 0.21
Saline	–/– only	5.2 ± 0.1	58 ± 6	0.25 ± 0.07	51 ± 15	0.388 ± 0.051	0.90 ± 0.13
48-h HI		5.5 ± 0.1	84 ± 9	0.51 ± 0.07	143 ± 23	0.840 ± 0.073	1.78 ± 0.18
Saline	All combined	5.1 ± 0.1	53 ± 5.1	0.28 ± 0.05	61 ± 13	0.416 ± 0.047	0.89 ± 0.11
Caucasians							
48-h HI		5.8 ± 0.1	55 ± 6	0.40 ± 0.04	98 ± 23	0.945 ± 0.059	2.01 ± 0.15
Saline	—	5.1 ± 0.1	37 ± 5	0.33 ± 0.05	79 ± 12	0.469 ± 0.036	1.07 ± 0.12
*P**							
Only –/–	Study	<0.0001	0.0002	0.0004	0.005	<0.0001	<0.0001
	Ethnicity	0.50	0.02	0.35	0.64	0.83	0.20
	Study × ethnicity	0.18	0.15	0.005	0.10	0.54	0.67
All	Study	<0.0001	0.0001	0.0001	0.002	<0.0001	<0.0001
	Ethnicity	0.15	0.02	0.20	0.55	0.49	0.02
	Study × ethnicity	0.22	0.24	0.003	0.07	0.98	0.45

Data are means ± SE or *P*. **P* value of ANOVA adjusted for BMI.

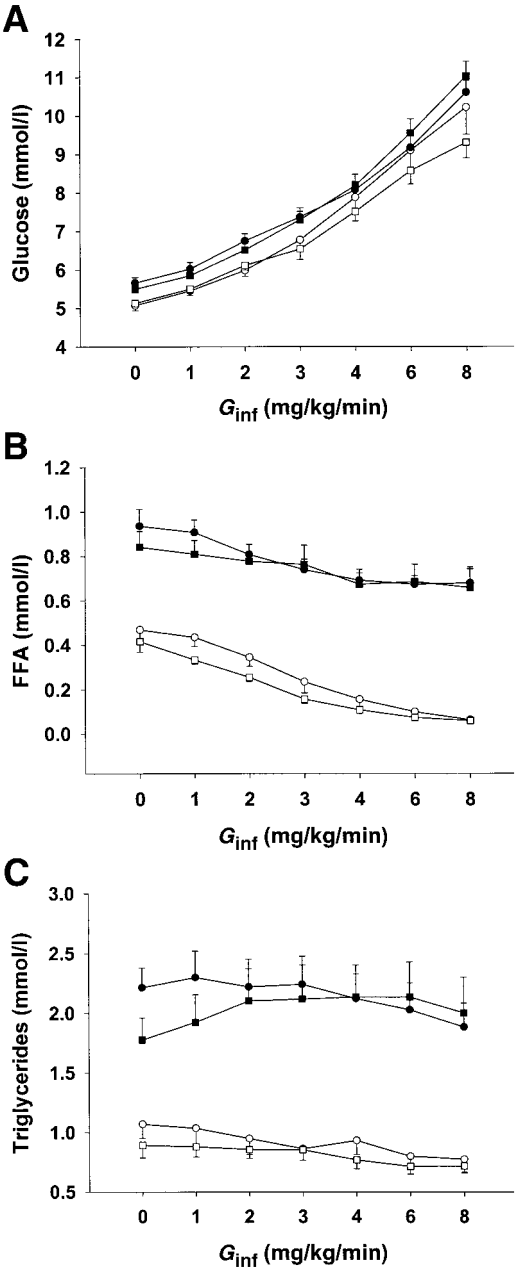

FIG. 2. Mean profiles of glucose (*A*), FFAs (*B*), and triglycerides (*C*) versus the glucose infusion step in response to a graded intravenous glucose infusion in Caucasian (○, ●; $n = 8$) and Oji-Cree individuals (□, ■; $n = 12$) during an intravenous infusion of saline (○, □) and after an intravenous infusion of HI for 48 h (●, ■). In the HI study, the infusion of heparin and Intralipid was continued throughout the experiment. The glucose, FFA, and triglycerides levels were higher in the HI versus saline study ($P < 0.0001$). The plasma FFA and triglyceride levels were slightly lower in Oji-Cree individuals than in Caucasian subjects ($P = 0.03$ and $P = 0.02$, respectively). Data are means ± SE. *P* values are after adjustment for BMI.

Plasma insulin, C-peptide, and ISR in response to glucose. In both ethnic groups, there was a clear shift to the left of the plasma insulin versus the glucose curve, with prolonged elevation of plasma FFA in Oji-Cree versus Caucasian subjects (Fig. 3*A* and *B*, respectively). The area under the insulin versus glucose curve between 6 and 9 mmol/l of plasma glucose was significantly higher after 48 h of HI than in the saline study ($P < 0.0001$) and was also significantly higher for the Oji-Cree group versus the Caucasian subjects ($P < 0.0001$). Furthermore, the mean increase of the area under the insulin versus glucose curve with HI infusion was higher in the Oji-Cree (70 vs. 21%, $P = 0.04$). The area under the C-peptide versus glucose curve (Fig. 3*C* and *D*) was also significantly higher in the Oji-Cree than in the Caucasian subjects ($P = 0.0001$) and was higher with HI infusion versus saline infusion only in the Oji-Cree ($P = 0.01$). In fact, there was a slight reduction (by ~5%) of the area under the C-peptide versus glucose curve with HI infusion in the Caucasian group, whereas it increased significantly by ~40% in the Oji-Cree group ($P < 0.0001$ for the difference of the change between the two ethnic groups). Similarly, the area under the ISR versus glucose curve increased by ~30% in the Oji-Cree group with HI, whereas it did not change in the Caucasian group (Fig. 3*E* and *F*) ($P = 0.04$ for difference of the change between the two ethnic groups). Excluding the three heterozygous carriers of the G319S mutation of the *HNF1α* gene did not change these results.

Insulin clearance. The insulin clearance rates during the graded glucose infusion studies were significantly lower in the Oji-Cree versus the Caucasian subjects ($P = 0.05$). Insulin clearance was reduced after prolonged elevation of plasma FFAs in the Caucasian group (Fig. 4*A*) but not in the Oji-Cree (Fig. 4*B*) ($P = 0.03$ for difference of the change between the two ethnic groups). These results did not change when the three heterozygous carriers of the G319S mutation of the *HNF1α* gene were excluded from the analyses.

The 20 mmol/l hyperglycemic clamp studies

Plasma glucose, insulin, C-peptide, FFAs, and triglycerides (Table 3). By design, plasma glucose levels during the hyperglycemic clamp were not different between the saline and the 48-h HI study or between the two ethnic groups. As expected, the plasma FFA and triglyceride levels were significantly higher in the 48-h HI study versus the saline study in both ethnic groups. Plasma FFA and triglyceride levels were not different between the two ethnic groups. The Oji-Cree participants had significantly higher plasma insulin levels than the Caucasian participants ($P = 0.008$) but similar C-peptide levels. Excluding the subjects carrying the G319S allele of the *HNF1α* gene did not change these results, except for the C-peptide results: one of the three heterozygous carriers of the G319S mutation had very high C-peptide levels during the hyperglycemic clamp, and excluding this subject resulted in a significant reduction of the mean plasma C-peptide level of the Oji-Cree group, below the level seen in Caucasian subjects ($P = 0.002$).

ISR, insulin clearance, $\mathbf{G_{inf}}$, $\mathbf{S_I}$, ***and*** $\mathbf{D_I}$. ISR (Fig. 5*A* and Table 3) were similar during the saline and the 48-h HI study and were similar between the two ethnic groups. However, excluding the three heterozygous carriers of the G319S mutation significantly reduced the ISR to below the levels seen in Caucasian subjects (to 317 ± 51 pmol/min in the saline study and to 407 ± 80 pmol/min in the HI study; $P = 0.02$ for difference with the Caucasian group). The marked difference seen after exclusion of the G319S mutation carriers was again solely because of a single outlier with very high C-peptide levels. Insulin clearance was not significantly affected by HI infusion during the 20 mmol/l hyperglycemic clamp, but was lower in the Oji-

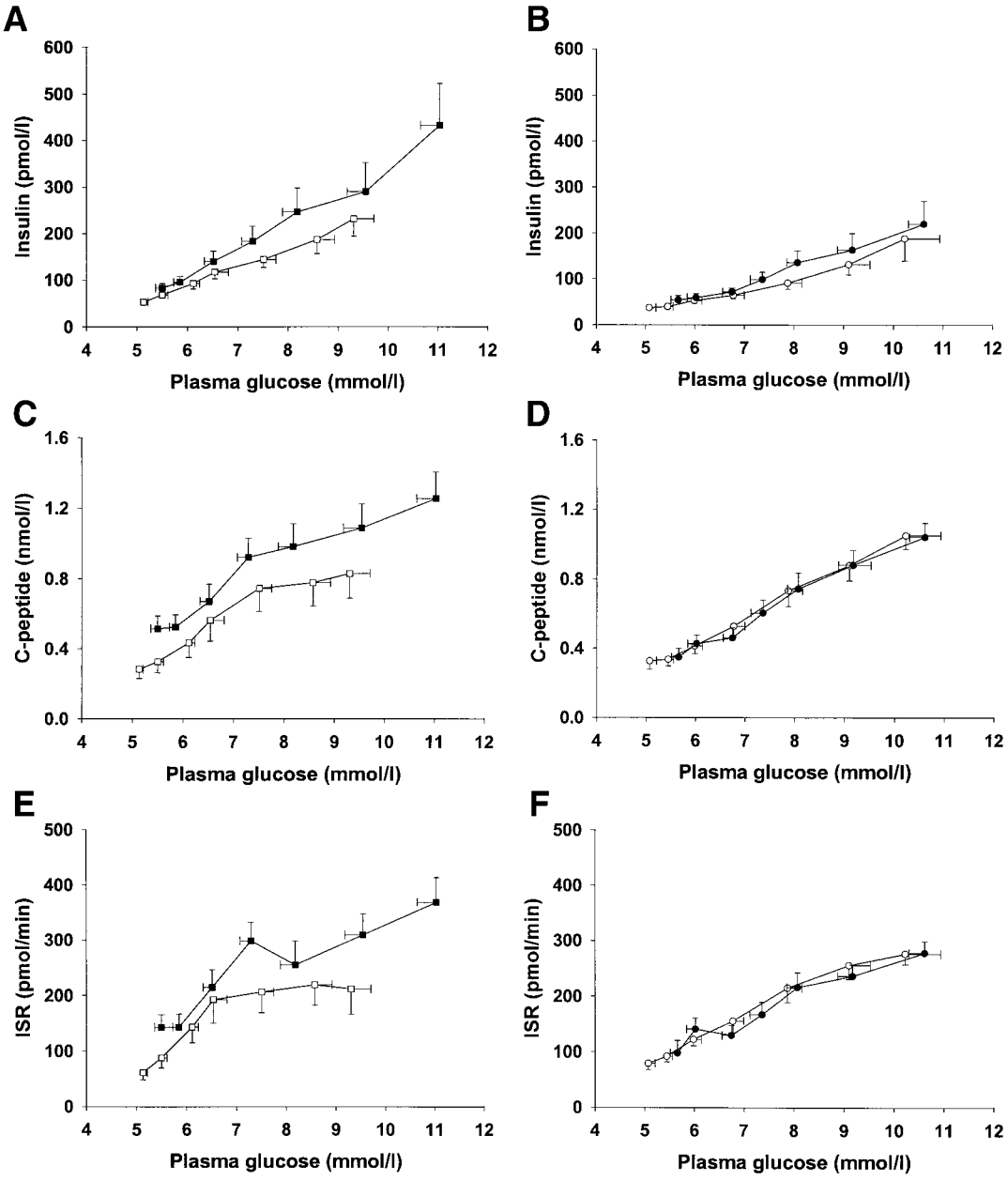

FIG. 3. Relationship between mean plasma insulin (*A* and *B*), C-peptide (*C* and *D*), and ISR (*E* and *F*) and mean plasma glucose level at each G_{inf} during the graded glucose infusion in the Oji-Cree individuals (*A*, *C*, and *E*; $n = 12$) and Caucasian subjects (*B*, *D*, and *F*; $n = 8$) and during intravenous saline (○, □) versus 48-h HI infusion (●, ■). The area under the insulin versus glucose curve between 6 and 9 mmol/l was significantly higher during the HI infusion ($P < 0.0001$) and was also higher in the Oji-Cree versus Caucasians ($P < 0.0001$). The area under the C-peptide versus glucose curve between 6 and 9 mmol/l was increased by 40% with infusion of HI for 48 h in the Oji-Cree, but was reduced by 5% in the Caucasian individuals (a significantly different response [$P = 0.0001$]). The area under ISR versus glucose curve between 6 and 9 mmol/l was increased by 30% by prolonged elevation of plasma FFAs in Oji-Cree individuals, whereas it did not change in Caucasians ($P = 0.04$ for the difference of response between the two groups). Data are means ± SE. *P* values are after adjustment for BMI.

Cree group compared with the Caucasian group only without inclusion of the heterozygous carriers of the G319S allele of the *HNF1α* gene ($P = 0.33$ with and $P < 0.0001$ without inclusion of the carriers of the G319S allele, respectively) (Fig. 5*B*). G_{inf} was significantly reduced to a similar extent (by 30–40%) in both ethnic groups with prolonged elevation of plasma FFA levels ($P = 0.0002$) (Fig. 5*C*), and this response was not changed by exclusion of the three heterozygous carriers of the G319S mutation ($P = 0.001$). S_I was also significantly reduced by ~40% in the 48-h HI study versus saline in both Oji-Cree and Caucasians ($P = 0.002$) (Fig. 5*D*). S_I was not lower in the Oji-Cree group ($P = 0.48$) when adjusted for BMI. However, when the three mutation carriers were excluded from the analysis, S_I was significantly lower in the Oji-Cree versus the Caucasians ($P = 0.007$). Mean D_I was lower in the Oji-Cree group than the Caucasian group by ~40%, but this difference was not significant when adjusted for BMI ($P = 0.82$), except when the three G319S allele carriers were removed from the analysis ($P < 0.0001$). After a 48-h HI infusion, D_I tended to be reduced in the whole group of subjects ($P = 0.05$) (Fig. 5*E*). When the three heterozygous carriers of the G319S mutation were not included in the analysis, D_I was significantly reduced by HI infusion ($P =$

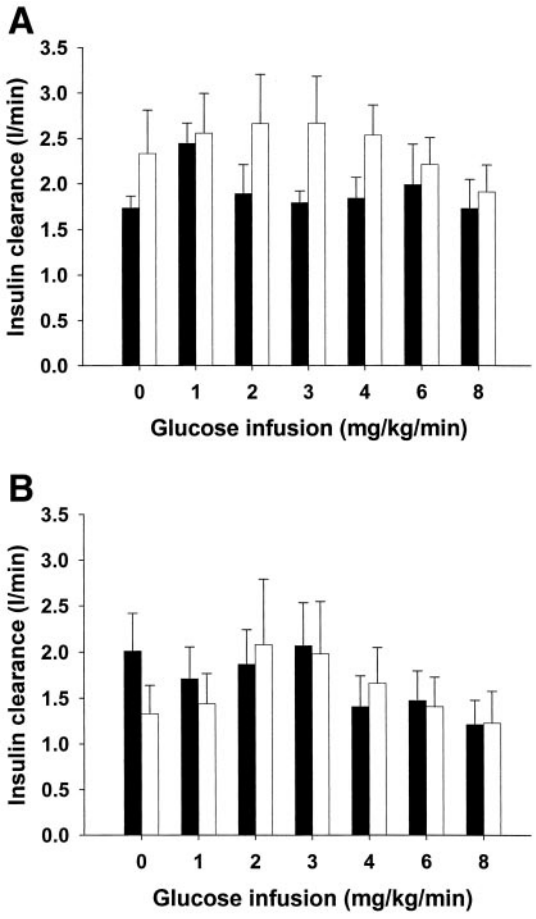

FIG. 4. Mean insulin clearance at each glucose infusion step during the intravenous graded glucose infusion study during saline (□) versus 48-h HI infusion (■) in Caucasian (*A*; *n* = 8) and Oji-Cree (*B*; *n* = 12) individuals. Although insulin clearance was reduced by prolonged HI infusion in Caucasians, it did not change in Oji-Cree individuals (*P* = 0.03 for the difference of response between the two groups). Data are means ± SE. *P* values are after adjustment for BMI.

0.003) over both groups and was significantly much lower in the Oji-Cree individuals than in the Caucasian subjects ($P < 0.0001$ vs. Caucasians). With adjustment for BMI, the reduction of D_I with HI infusion was also significantly lower in the Oji-Cree than in the Caucasians only with exclusion of the three heterozygous carriers of the G319S mutation ($P = 0.39$ without and $P = 0.04$ with exclusion of these individuals). Although the reduction of D_I with prolonged elevation of plasma FFAs was striking in the Caucasian group (by ~40%), this reduction was only ~15% in the Oji-Cree group ($P = 0.03$ for difference of response between the Oji-Cree and the Caucasians).

DISCUSSION

In the present study, we examined the effect of a prolonged infusion of heparin and Intralipid for 48 h, which resulted in an approximately twofold elevation of plasma FFAs above fasting levels, in Oji-Cree individuals with normal glucose tolerance from the Sandy Lake community of Ontario, a population with a very high risk of developing type 2 diabetes. The elevation of FFAs was associated with an absolute increase in GSIS, but, because there was a concomitant 40% reduction in S_I, the D_I was marginally reduced by 15%. In other words, the pancreatic β-cell was unable to adequately compensate for the FFA-induced reduction in S_I by hypersecreting insulin—a finding that can be interpreted as an FFA-mediated impairment of β-cell function. Furthermore, this slight reduction of D_I with prolonged elevation of plasma FFAs in the Oji-Cree individuals appears to be independent of a reduction in insulin clearance. The important and surprising finding, however, was that although the reduction in S_I seen in these subjects with prolonged elevation of plasma FFA was of similar magnitude to that seen in Caucasian individuals, the reduction of D_I was much less than that seen in Caucasians (40%). We had originally anticipated finding greater, not lesser, impairment of β-cell function with FFA elevation in this population, in which rapid changes in diet and physical activity on a susceptible genetic background have provoked an epidemic of type 2 diabetes in recent years. It is important to note, however, that S_I and D_I in the Oji-Cree individuals was already very low before the infusion of Intralipid and heparin compared with the Caucasian population, in keeping with the very high risk of developing diabetes in this population (31). Therefore, the different insulin secretory response to FFAs in the former group may only reflect the nonlinearity of the association between S_I and ISR and/or a floor effect.

There are three possible interpretations for the apparent lesser susceptibility to FFA-mediated desensitization of GSIS seen in this group with normal glucose tolerance: *1*) an intrinsic, possibly genetically determined, resistance to β-cell lipotoxicity, a term originally coined by Roger Unger to describe the fatty acid–induced impairment of pancreatic β-cell function (8); *2*) an already present deleterious lipotoxic effect from previous excess exposure of β-cells to fatty acids; or *3*) the fact that the reduction of D_I induced by prolonged elevation of plasma FFAs is, at least partly, an adaptive response triggered by a reduction of hepatic insulin clearance that does not occur in Oji-Cree individuals because they do not display FFA-induced reduction of insulin clearance. Although the first possibility cannot be ruled out at the present time, we speculate that it is less likely for the following reasons. First, historical reports from the Sandy Lake community suggest that type 2 diabetes was almost nonexistent in the first part of the 20th century and that the very high incidence and prevalence of this disease followed the introduction of high-fat food and a sedentary lifestyle in this community (32). This fact suggests that these individuals are highly susceptible to environmental changes that have been associated with a high risk for the development of type 2 diabetes. Second, although the reduction of D_I was modest, it was already very low in the Oji-Cree group. It is therefore possible that a modest 15% reduction in D_I may nevertheless have a significant impact on glucose homeostasis at this level of impaired β-cell function. One possible mechanism to maintain normal glucose homeostasis in the face of a reduced S_I could be a reduction in insulin clearance. However, this did not occur in the Oji-Cree individuals with HI infusion, a phenomenon that we also observed previously in Caucasian individuals with type 2 diabetes (17). Once again, insulin clearance was already reduced in the Oji-Cree individuals before the infusion of heparin and Intralipid. The higher glucose levels during the graded intravenous glucose infusion after

TABLE 3
Mean levels during the last 30 min of the 20 mmol/l hyperglycemic clamp

Ethnicity Study	G319S HNF1α	Glucose (mmol/l)	Insulin (pmol/l)	C-peptide (nmol/l)	ISR (pmol/min)	FFAs (mmol/l)	Triglycerides (mmol/l)
Oji-cree							
48-h HI		20.3 ± 0.4	1,680 ± 241	1.57 ± 0.20	407 ± 80	0.619 ± 0.092	2.02 ± 0.31
Saline	–/–	20.2 ± 0.2	1,335 ± 214	1.20 ± 0.18	317 ± 51	0.052 ± 0.008	0.78 ± 0.08
48-h HI		20.1 ± 0.4	1,434 ± 227	2.10 ± 0.44	518 ± 99	0.599 ± 0.071	1.81 ± 0.27
Saline	All combined	20.1 ± 0.2	1,179 ± 187	1.83 ± 0.51	511 ± 172	0.047 ± 0.006	0.72 ± 0.07
Caucasians							
48-h HI		20.0 ± 0.5	649 ± 50	2.13 ± 0.22	541 ± 54	0.585 ± 0.065	1.60 ± 0.29
Saline	—	19.7 ± 0.4	585 ± 47	2.02 ± 0.19	551 ± 57	0.018 ± 0.005	0.53 ± 0.07
*P**							
Only –/–	Study	0.64	0.22	0.23	0.53	<0.0001	<0.0001
	Ethnicity	0.46	0.005	0.002	0.02	0.38	0.35
	Study × ethnicity	0.91	0.40	0.51	0.44	1.00	0.70
All	Study	0.69	0.29	0.67	0.99	<0.0001	<0.0001
	Ethnicity	0.84	0.008	0.80	0.78	0.66	0.67
	Study × ethnicity	0.60	0.53	0.86	0.95	0.88	0.96

Data are means ± SE or *P*. **P* value of ANOVA adjusted for BMI.

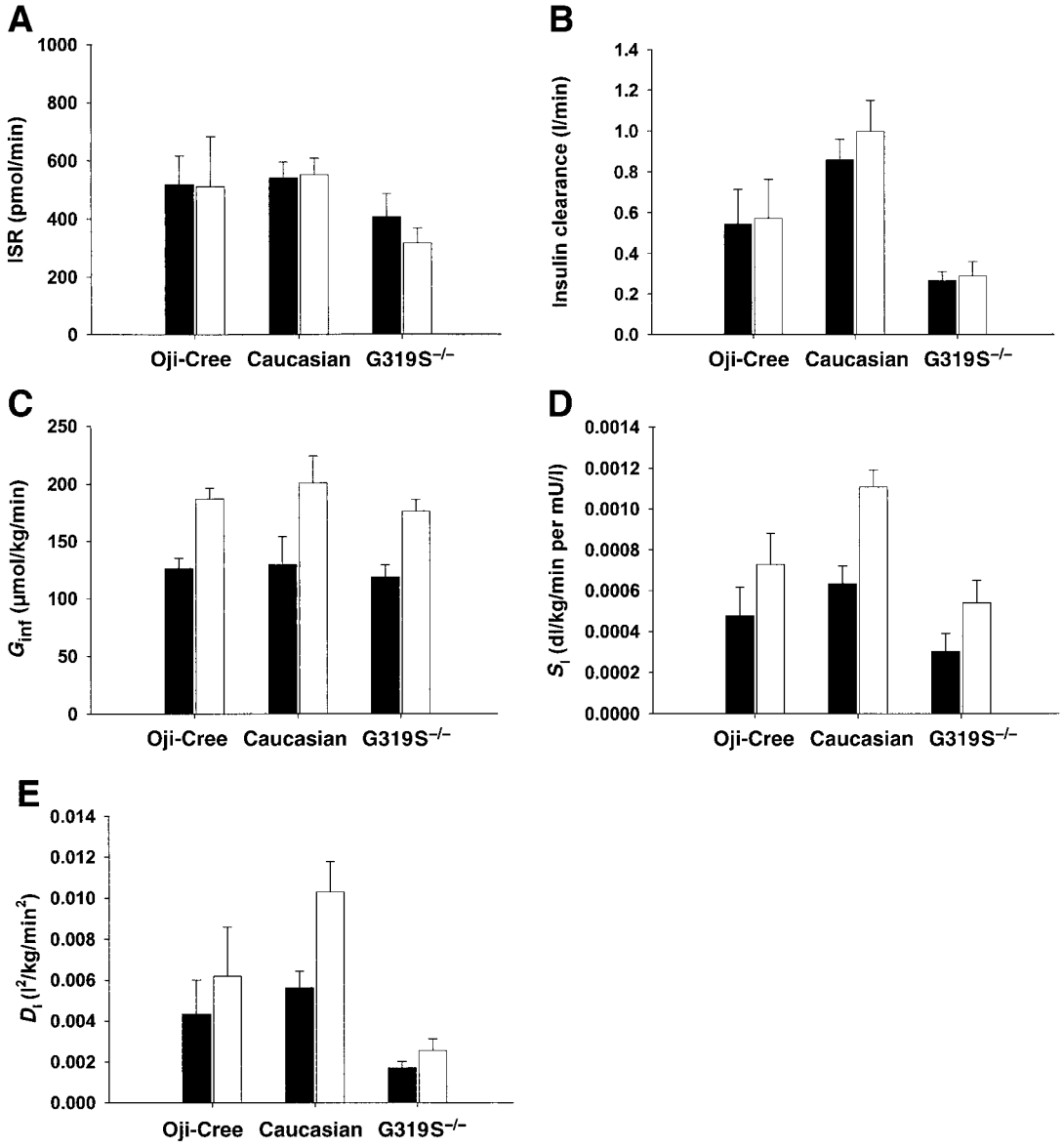

FIG. 5. ISR (*A*), insulin clearance (*B*), G_{inf} (*C*), S_I (*D*), and D_I (*E*) from the 20 mmol/l hyperglycemic clamp study during saline (□) versus 48-h HI infusion (■). Data including (Oji-Cree, $n = 12$) and excluding (G319S$^{-/-}$, $n = 9$) the three Oji-Cree carriers of the G319S mutation of the *HNF1α* gene are shown in comparison to those of the Caucasian group ($n = 8$). The 48-h HI infusion did not change ISR in both groups. ISRs were similar between the two ethnic groups when examined in the whole group but were lower in Oji-Cree individuals when the carriers of the G319S mutation were excluded ($P = 0.02$). The 48-h HI infusion also did not change insulin clearance but was reduced in the Oji-Cree individuals versus Caucasians only with exclusion of the three mutation carriers ($P < 0.0001$). The G_{inf} during the clamp was reduced by 30–40% in both groups after a 48-h elevation of plasma FFAs ($P = 0.0002$). Exclusion of the three heterozygous carriers of the G319S mutation did not modify this response ($P = 0.001$). Accordingly, the clamp S_I was also significantly reduced by ~40% in the 48-h HI study versus saline in both Oji-Cree and Caucasians ($P = 0.002$). Insulin sensitivity was not significantly lower in Oji-Cree versus Caucasians ($P = 0.48$) when examined in the whole group, but was significantly lower versus the Caucasians when the three mutation carriers were excluded from the analysis ($P = 0.007$). Mean D_I was not significantly lower in the Oji-Cree group versus the Caucasian group when examined in the whole group, but tended to be lower in the HI study versus the saline study ($P = 0.05$). When the carriers of the G319S mutation were excluded from the analysis, D_I was significantly reduced by HI infusion ($P = 0.003$) and was significantly much lower in the remaining Oji-Cree individuals than in the Caucasians ($P < 0.0001$). Furthermore, the reduction of D_I with prolonged elevation of plasma FFAs was significantly lower in the Oji-Cree (15%) than in the Caucasian (40%) individuals ($P = 0.04$). Data are means ± SE. *P* values are after adjustment for BMI.

HI versus saline infusion in the Oji-Cree individuals is also in keeping with the interpretation of a reduced adaptation of insulin secretion to the ambient degree of insulin resistance when FFAs are elevated.

Fasting plasma FFA and triglyceride levels were not elevated at baseline in the Oji-Cree individuals, which does not support antecedent excessive exposure of their pancreatic β-cells to fatty acids. However, the in vivo exposure of β-cells to fatty acids may not be solely determined by fasting plasma FFA levels, but may perhaps be a function of postprandial fat partitioning between the adipose tissue and the other organs (33). The significant elevation of ISR with prolonged elevation of plasma FFAs may be regarded as a normal adaptive response of β-cells to the FFA-mediated reduction in S_I. However, we have previously observed a similar response in patients with established type 2 diabetes (17). Therefore, an established reduction of GSIS is not necessarily associated with a complete loss of the adaptive capacity of the β-cells to elevated plasma FFAs. More studies are clearly needed in

Oji-Cree individuals and in other populations at very high risk of developing type 2 diabetes to further address the determinants of FFA-mediated desensitization of GSIS in vivo.

The reason for the lower plasma FFA and triglyceride levels during the graded intravenous glucose infusion study in the Oji-Cree versus Caucasian group is not clear, because our study was not designed to assess plasma FFA and triglyceride metabolism. The higher plasma insulin levels may explain the lower plasma FFA levels in the former group because insulin potently inhibits plasma FFA levels and appearance rate (33). Further studies will be needed to assess whether a reduced secretion rate and/or improved clearance of triglyceride-rich lipoproteins is present in Oji-Cree individuals.

Previous studies by some members of our group have revealed that a private mutation (G319S) of the *HNF1α* gene explains a large proportion of the diabetes in the Oji-Cree population of the Sandy Lake region, but that unaffected individuals still have a very high prevalence of type 2 diabetes. Approximately 25% of subjects are heterozygous carriers of the G319S mutation of the *HNF1α* gene (21). Heterozygosity for this mutation confers a ~97% risk of developing glucose intolerance by the age of 50, whereas the risk in noncarriers in this population is still very high at ~42% (22). Furthermore, this mutation has been shown to reduce hepatic nuclear factor-1α–mediated gene transcription in vitro by 50% and is associated with reduction of the plasma insulin levels in this population (34). Of the 12 Oji-Cree participants in our study, 3 were heterozygous carriers of this mutation, in keeping with the prevalence of the mutation in this population. The recruitment of carriers of this mutation who had normal glucose tolerance and were willing to spend a week in Toronto to participate in our study was extremely challenging, and we were unable to further increase the sample size over a prolonged period. Because of these recruitment difficulties, our capacity to draw conclusions regarding the effect of prolonged elevation of plasma FFAs in carriers of the G319S mutation was limited. Nevertheless, keeping these three individuals in our group of participants makes the group representative of the population of the Sandy Lake region (25% prevalence of the G319S mutation). Furthermore, analyzing the data with or without these individuals did not change our major conclusion that Oji-Cree individuals do not appear to be more susceptible to FFA-mediated desensitization of GSIS. It is interesting to note that the G319S mutation significantly reduces the age of onset of diabetes in that community in women only, not in men (35). This sex difference could be at least partly explained by the higher prevalence of obesity in Oji-Cree women (32,35). Therefore, there is evidence that this mutation interacts with classic risk factors to induce the development of type 2 diabetes.

In conclusion, glucose-tolerant healthy Oji-Cree individuals from the Sandy Lake region, a population at very high risk of developing type 2 diabetes, display reduced susceptibility to desensitization of GSIS by prolonged in vivo elevation of plasma FFAs compared with healthy Caucasian individuals. However, these individuals have lower insulin sensitivity and D_I at baseline than Caucasians. Whether this different response between Oji-Cree and Caucasian individuals reflects an already-present lipotoxic effect or an inherent resistance to FFA-induced β-cell dysfunction in the former will require further studies.

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REFERENCES

1. Taylor SI, Accili D, Imai Y: Insulin resistance or insulin deficiency: which is the primary cause of NIDDM? *Diabetes* 43:735–740, 1994
2. DeFronzo RA, Bonadonna RC, Ferrannini E: Pathogenesis of NIDDM: a balanced overview. *Diabetes Care* 15:318–368, 1992
3. Porte D Jr: Banting lecture 1990: beta-cells in type II diabetes mellitus. *Diabetes* 40:166–180, 1991
4. Polonsky KS: Lilly Lecture 1994: the beta-cell in diabetes: from molecular genetics to clinical research. *Diabetes* 44:705–717, 1995
5. Sako Y, Grill VE: A 48-hour lipid infusion in the rat time-dependently inhibits glucose-induced insulin secretion and B cell oxidation through a process likely coupled to fatty acid oxidation. *Endocrinology* 127:1580–1589, 1990
6. Ohneda M, Inman LR, Huger RH: Caloric restriction in obese pre-diabetic rats prevents beta-cell depletion, lost of beta-cell GLUT2 and glucose incompetence. *Diabetologia* 38:173–179, 1995
7. Capito K, Hansen SE, Hedeskov CJ, Islin H, Thams P: Fat-induced changes in mouse pancreatic islet insulin secretion, insulin biosynthesis and glucose metabolism. *Acta Diabetol* 28:193–198, 1992
8. Unger RH: Lipotoxicity in the pathogenesis of obesity-dependent NIDDM: genetic and clinical implications. *Diabetes* 44:863–870, 1995
9. Prentki M, Corkey BE: Are the beta-cell signaling molecules malonyl-CoA and cytosolic long-chain acyl-CoA implicated in multiple tissue defects of obesity and NIDDM? (Review) *Diabetes* 45:273–283, 1996
10. Charles MA, Eschwège E, Thibault N, Claude JR, Warnet JM, Rosselin GE, Girard J, Balkau B: The role of non-esterified fatty acids in the deterioration of glucose tolerance in Caucasian subjects: results of the Paris Prospective Study. *Diabetologia* 40:1101–1106, 1997
11. Knowler WC, Pettitt DJ, Saad MF, Bennett PH: Diabetes mellitus in the Pima Indians: incidence, risk factors and pathogenesis. *Diabete Metab Rev* 6:1–27, 1990
12. Mason TM, Goh T, Tchipashvili V, Sandhu H, Gupta N, Lewis GF, Giacca A: Prolonged elevation of plasma free fatty acids desensitizes the insulin secretory response to glucose in vivo in rats. *Diabetes* 48:524–530, 1999
13. Dobbins RL, Szczepaniak LS, Myhill J, Tamura Y, Uchino H, Giacca A, McGarry JD: The composition of dietary fat directly influences glucose-stimulated insulin secretion in rats. *Diabetes* 51:1825–1833, 2002
14. Paolisso G, Gambardella A, Amato L, Tortoriello R, D'Amore A, Varrichio M, D'Onofrio F: Opposite effects of short- and long-term fatty acid infusion on insulin secretion in healthy subjects. *Diabetologia* 38:1295–1299, 1996
15. Paolisso G, Tagliamonte MR, Rizzo MR, Gualdiero P, Saccomanno F, Gambardella A, Giugliano D, D'Onofrio F, Howard BV: Lowering fatty acids potentiates acute insulin response in first degree relatives of people with type II diabetes. *Diabetologia* 41:1127–1132, 1998
16. Carpentier A, Mittelman SD, Lamarche B, Bergman RN, Giacca A, Lewis GF: Acute enhancement of insulin secretion by FFA in humans is lost with prolonged FFA elevation. *Am J Physiol* 276:E1055–E1066, 1999
17. Carpentier A, Mittelman SD, Bergman RN, Giacca A, Lewis GF: Prolonged elevation of plasma free fatty acids impairs pancreatic β-cell function in obese nondiabetic humans but not in individuals with type 2 diabetes. *Diabetes* 49:399–408, 2000
18. Boden G, Chen X: Effects of fat on glucose uptake and utilization in patients with non-insulin-dependent diabetes. *J Clin Invest* 96:1261–1268, 1995
19. Haffner SM: Epidemiology at type 2 diabetes: risk factors. *Diabetes Care* 21:C3–C6, 1998
20. Harris MI, Flegal KM, Cowie CC, Eberhardt MS, Goldstein DE, Little RR, Wiedmeyer HM, ByrdHolt DD: Prevalence of diabetes, impaired fasting glucose, and impaired glucose tolerance in U.S. adults: the Third National

Health and Nutrition Examination Survey, 1988–1994. *Diabetes Care* 21:518–524, 1998
21. Hegele RA, Cao H, Harris SB, Hanley AJ, Zinman B: The hepatic nuclear factor-1alpha G319S variant is associated with early-onset type 2 diabetes in Canadian Oji-Cree. *J Clin Endocrinol Metab* 84:1077–1082, 1999
22. Hegele RA, Cao H, Hanley AJ, Zinman B, Harris SB, Anderson CM: Clinical utility of HNF1α genotyping for diabetes in aboriginal Canadians. *Diabetes Care* 23:775–778, 2000
23. Gittelsohn J, Wolever TM, Harris SB, Harris-Giraldo R, Hanley AJ, Zinman B: Specific patterns of food consumption and preparation are associated with diabetes and obesity in a Native Canadian community. *J Nutr* 128:541–547, 1998
24. Expert Committee on the Diagnosis and Classification of Diabetes Mellitus: Report of the Expert Committee on the Diagnosis and Classification of Diabetes Mellitus. *Diabetes Care* 20:1183–1197, 1997
25. DeFronzo RA, Tobin JD, Andres R: Glucose clamp technique: a method for quantifying insulin secretion and resistance. *Am J Physiol* 237:E214–E223, 1979
26. Van Cauter E, Mestrez F, Sturis J, Polonsky KS: Estimation of insulin secretion rates from C-peptide levels: comparison of individual and standard kinetic parameters for C- peptide clearance. *Diabetes* 41:368–377, 1992
27. Balent B, Goswami G, Goodloe G, Rogatsky E, Rauta O, Nezami R, Mints L, Angeletti RH, Stein DT: Acute elevation of NEFA causes hyperinsulinemia without effect on insulin secretion rate in healthy human subjects. *Ann N Y Acad Sci* 967:535–543, 2002
28. Bergman RN, Finegood DT, Ader M: Assessment of insulin sensitivity in vivo. *Endocr Rev* 6:45–86, 1985
29. Kahn SE, Prigeon RL, McCulloch DK, Boyko EJ, Bergman RN, Schwartz MW, Neifing JL, Ward WK, Beard JC, Palmer JP, Porte DJ: Quantification of the relationship between insulin sensitivity and β-cell function in human subjects: evidence for a hyperbolic function. *Diabetes* 42:1663–1672, 1993
30. Byrne MM, Sturis J, Polonsky KS: Insulin secretion and clearance during low-dose graded glucose infusion. *Am J Physiol* 268:E21–E27, 1995
31. Weyer C, Bogardus C, Mott DM, Pratley RE: The natural history of insulin secretory dysfunction and insulin resistance in the pathogenesis of type 2 diabetes mellitus. *J Clin Invest* 104:787–794, 1999
32. Harris SB, Gittelsohn J, Hanley A, Barnie A, Wolever TM, Gao J, Logan A, Zinman B: The prevalence of NIDDM and associated risk factors in native Canadians. *Diabetes Care* 20:185–187, 1997
33. Lewis GF, Carpentier A, Adeli K, Giacca A: Disordered fat storage and mobilization in the pathogenesis of insulin resistance and type 2 diabetes. *Endocr Rev* 23:201–229, 2002
34. Triggs-Raine BL, Kirkpatrick RD, Kelly SL, Norquay LD, Cattini PA, Yamagata K, Hanley AJ, Zinman B, Harris SB, Barrett PH, Hegele RA: HNF-1alpha G319S, a transactivation-deficient mutant, is associated with altered dynamics of diabetes onset in an Oji-Cree community. *Proc Natl Acad Sci U S A* 99:4614–4619, 2002
35. Hegele RA, Cao H, Harris SB, Zinman B, Hanley AJ, Anderson CM: Gender, obesity, hepatic nuclear factor-1alpha G319S and the age-of-onset of type 2 diabetes in Canadian Oji-Cree. *Int J Obes Relat Metab Disord* 24:1062–1064, 2000