

UC Davis

UC Davis Previously Published Works

Title

Parasympathetic Blockade Attenuates Augmented Pancreatic Polypeptide But Not Insulin Secretion in Pima Indians

Permalink

<https://escholarship.org/uc/item/890485z2>

Journal

Diabetes, 53(3)

ISSN

0012-1797

Authors

de Courten, Barbora Vozarova
Weyer, Christian
Stefan, Norbert
[et al.](#)

Publication Date

2004-03-01

DOI

10.2337/diabetes.53.3.663

Peer reviewed

Parasympathetic Blockade Attenuates Augmented Pancreatic Polypeptide But Not Insulin Secretion in Pima Indians

Barbora Vozarova de Courten,¹ Christian Weyer,¹ Norbert Stefan,¹ Mark Horton,² Angelo DelParigi,¹ Peter Havel,³ Clifton Bogardus,¹ and P. Antonio Tataranni¹

There is evidence from animal models of obesity and type 2 diabetes that increased parasympathetic vagal input to the pancreas contributes to hyperinsulinemia. Compared with Caucasians, Pima Indians have a high risk of type 2 diabetes and exhibit marked hyperinsulinemia and elevated plasma levels of pancreatic polypeptide (PP), an islet hormone considered a surrogate marker of parasympathetic nervous system (PNS) drive to the pancreas. To test if hyperinsulinemia in Pima Indians is due to increased vagal input to the β -cell, we examined the effect of PNS blockade in 17 Caucasian (aged 35 ± 8 years, body fat $23 \pm 7\%$ [mean \pm SD]) and 17 Pima Indian males (aged 28 ± 8 years, body fat $29 \pm 5\%$) with normal glucose tolerance. Each participant underwent four consecutive standardized liquid meal tests (64% carbohydrate, 22% fat, and 14% protein) during which a primed infusion of atropine was administered for 120 min at the following doses: 0, 2.5, 5, and 10 $\mu\text{g} \cdot \text{kg fat-free mass (FFM)}^{-1} \cdot \text{h}^{-1}$. Areas under the curve for early ($\text{AUC}_{0-30 \text{ min}}$) and total ($\text{AUC}_{0-120 \text{ min}}$) postprandial insulin and PP secretory responses were calculated. Early postprandial insulin and PP secretory responses were higher in Pima Indians compared with those of Caucasians (both $P = 0.01$). Secretion of insulin and PP was inhibited by atropine (both $P < 0.001$). Increasing doses of atropine attenuated the ethnic difference in PP ($P = 0.01$) but not in early insulin secretory responses ($P = 0.6$), an effect that was not due to differences in gastric emptying rate (acetaminophen test) and/or circulating glucose. Similar results were observed for total secretory responses. These results confirm that compared with Caucasians, Pima Indians have an exaggerated PNS drive to pancreatic F-cells that secrete PP. However, the hyperinsulinemia of this population does not appear to be due to increased vagal input to pancreatic β -cells. *Diabetes* 53: 663–671, 2004

From the ¹Clinical Diabetes and Nutrition Section, National Institute of Diabetes and Digestive and Kidney Diseases, National Institutes of Health, Phoenix, Arizona; the ²Ophthalmology Department, Phoenix Indian Medical Center, Phoenix, Arizona; and the ³Department of Nutrition, University of California–Davis, Davis, California.

Address correspondence to P. Antonio Tataranni, MD, Clinical Diabetes and Nutrition Section, National Institutes of Health, 4212 N. 16th St., Rm. 5-41, Phoenix, AZ 85016. E-mail: antoniot@mail.nih.gov.

Received for publication 13 August 2003 and accepted in revised form 17 November 2003.

AUC, area under the curve; FFM, fat-free mass; PNS, parasympathetic nervous system; PP, pancreatic polypeptide.

© 2004 by the American Diabetes Association.

The Pima Indians of Arizona have one of the highest reported prevalence rates of obesity and type 2 diabetes in the world (1). Compared with Caucasians, Pima Indians have, on average, lower insulin sensitivity and higher plasma insulin concentrations, both fasting and in response to intravenous and oral glucose loads (2). Prospectively, fasting hyperinsulinemia predicts the development of diabetes in both Pima Indians (3–5) and other populations (6,7). The hyperinsulinemia in Pima Indians appears to be an early characteristic because Pima Indian children have 50–70% higher fasting insulin concentrations than Caucasian children (8).

The precise mechanisms contributing to hyperinsulinemia in Pima Indians remain unknown. In part, the higher fasting insulin concentrations may be a secondary adaptation of the β -cell to compensate for the high degree of insulin resistance. However, the latter does not appear to entirely account for the hyperinsulinemia (2).

Hyperinsulinemia is also an early feature of various animal models of both genetic and hypothalamic obesity and type 2 diabetes, such as the *ob/ob* mouse (9), the *fa/fa* rat (10), and rodents with lesions of the ventromedial hypothalamus (11,12). In these animals, the hyperinsulinemia is thought to be due in large part to an exaggerated parasympathetic drive to the pancreatic β -cells because acute administration of parasympatholytic drugs such as atropine (a blocker of muscarinic receptors) (9–11) or truncal vagotomy (12,13) can almost completely normalize insulin levels.

Compared with Caucasians, Pima Indians have been reported to have higher plasma concentrations of pancreatic polypeptide (PP) both in the fasting state and especially in response to a meal (14,15). PP is a peptide hormone produced by the pancreatic F-cells, the secretion of which is largely under vagal control (16). In response to a meal, plasma PP concentrations increase, in parallel to an increase in plasma insulin concentrations (15,17). Vagotomy (17) or administration of parasympatholytic drugs such as atropine completely abolish the postprandial increase in PP in humans (18,19). Conversely, electrical stimulation of the vagus nerve or administration of parasympathomimetic drugs such as pyridostigmin (a cholinesterase inhibitor) increase PP secretion (19,20). Based on these findings, it is generally agreed that the plasma PP

Experimental protocol

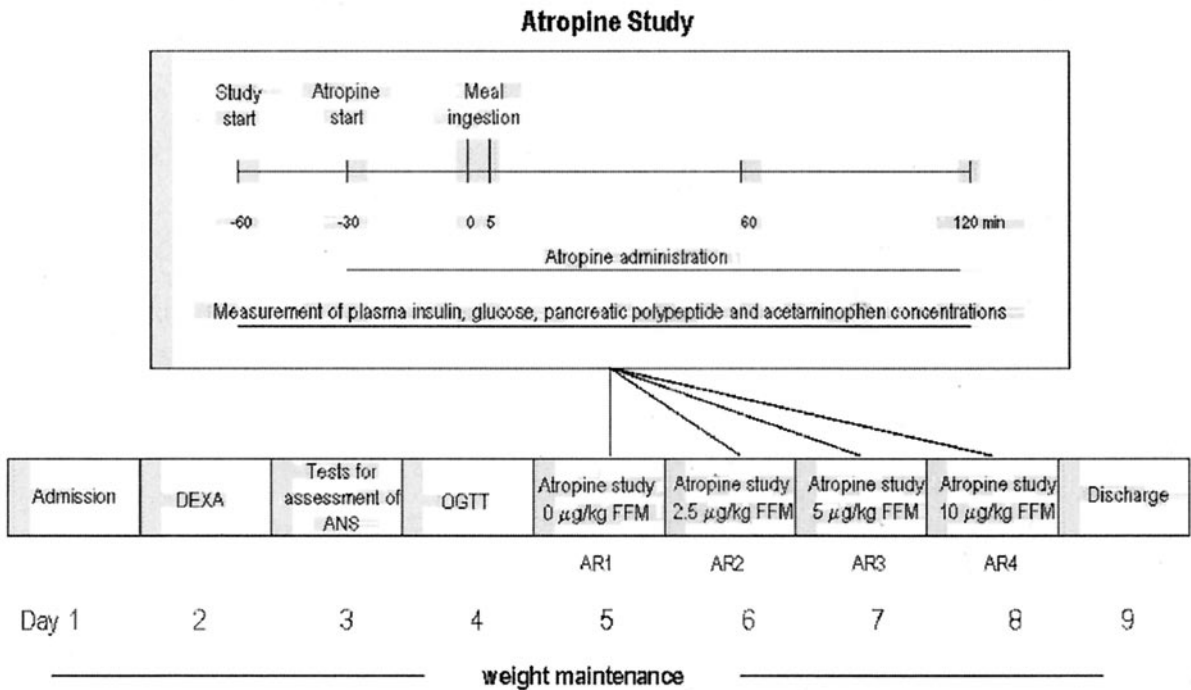


FIG. 1. Study design. Subjects admitted to the Clinical Research Unit first underwent a series of four standard autonomic nervous system (ANS) tests and a 75-g oral glucose tolerance test (OGTT). Then, on 4 separate days, four atropine infusion studies were conducted. In each of the studies, after 30 min baseline, an atropine infusion was administered at the following doses: 0, 2.5, 5 and 10 $\mu\text{g} \cdot \text{kg FFM}^{-1} \cdot \text{h}^{-1}$ and continued until the end of the experiment. At 60 min (30 min after the start of atropine administration), a liquid meal test was administered. Plasma PP, insulin, glucose, acetaminophen, blood pressure, and heart rate were measured throughout the experiment. DEXA, dual-energy X-ray absorptiometry.

concentration represents a valid surrogate marker of the parasympathetic drive to the pancreas (16).

Based on these findings, we hypothesized that an increased parasympathetic drive to the pancreas might contribute to hyperinsulinemia in Pima Indians. To test this hypothesis, we examined the effect of graded doses of the parasympathetic nervous system (PNS) blocker atropine on postprandial PP and insulin secretory responses in 17 Caucasian and 17 Pima Indian men with normal glucose tolerance.

RESEARCH DESIGN AND METHODS

Subjects. Seventeen Caucasian and 17 Pima Indian men were admitted for 7–10 days to the metabolic ward of the Clinical Diabetes and Nutrition Section of the National Institutes of Health in Phoenix, AZ. Women were not studied to avoid the confounding effects of hormonal changes during the menstrual cycle. All subjects were between 18 and 50 years of age, normal glucose tolerant according to a 75-g oral glucose tolerance test (World Health Organization 1985 criteria) (21), nonsmokers at the time of the study, and, except for obesity, healthy according to a physical examination and routine laboratory tests. None were taking medications at the time of entry into the study. The protocol was approved by the Institutional Review Boards of the National Institute of Diabetes and Digestive and Kidney Diseases and the Phoenix area. All subjects provided written informed consent before participation.

Methods and experimental protocol. Subjects were admitted for 8–10 days to the National Institutes of Health Clinical Research Unit in Phoenix, AZ, where they were fed a weight-maintaining diet (50, 30, and 20% of daily calories provided as carbohydrate, fat, and protein, respectively), and abstained from strenuous exercise.

Body composition was estimated by total body dual-energy X-ray absorptiometry (DPX-L; Lunar Radiation, Madison, WI) with calculations of percentage of body fat, fat mass, and fat-free mass (FFM) as previously described (22).

At least 3 days after admission and after a 12-h overnight fast, subjects underwent a 2-h 75-g oral glucose tolerance test. Plasma glucose concentrations were determined by the glucose oxidase method (Beckman Instruments, Fullerton, CA) and plasma insulin concentrations by an automated immunoassay (Access; Beckman Instruments).

On day 3, before administration of atropine, subjects underwent a series of four standard tests for the assessment of cardiovascular autonomic nervous system activity (23). During these tests, subjects were in a sitting position and blood pressure and electrocardiogram were monitored continuously. In the first two tests, which assessed PNS activity, the change in heart rate was assessed in response to deep breathing (the difference between minimum and maximum heart rate during six deep breathing cycles per min) and a Valsalva maneuver (the ratio of the longest R-R interval after the maneuver to the shortest R-R interval during the maneuver). In the third and fourth tests, which assessed sympathetic nervous system activity, the change in blood pressure was measured in response to standing up (the difference between the lowest systolic pressure in the first 2 min after standing up and the average systolic pressure while lying down) and in response to a hand-grip test (using a dynamometer, 30% of maximum voluntary contraction over 5 min).

On 4 separate days after a 12-h overnight fast, subjects were fed a standardized liquid meal (Ensure Plus, 20% of daily caloric requirements [64% carbohydrate, 22% fat, and 14% protein, identical on all 4 days]) (Fig. 1). The room was maintained at mesopic illumination levels, and the volunteer was instructed to abstain from near-centered visual tasks. During each test, two intravenous catheters were placed in an antecubital vein on the left and right arm, one for the saline/atropine infusion and one for blood sampling. After a 30-min baseline period, a primed-continuous infusion of the study medication was started and maintained for 150 min. To avoid the risk of untoward effects of high-dose atropine in hypersensitive individuals, infusions were not randomized. The infusions contained saline on the first occasion, always followed by three increasing doses of atropine. Atropine was administered in doses of 2.5, 5, or 10 $\mu\text{g}/\text{kg FFM}$ for priming (injected over 2 min), followed by a continuous infusion at doses of 2.5, 5, or 10 $\mu\text{g} \cdot \text{kg FFM}^{-1} \cdot \text{h}^{-1}$, respectively. At 0 min, subjects ingested the standardized liquid meal within 5 min. Since atropine was shown to affect gastrointestinal motility, we estimated the rate of gastric emptying by use of the acetaminophen absorption test (24,25). For this

TABLE 1
Baseline physical and metabolic characteristics of 17 Pima Indians and 17 Caucasians

	Pima Indians	Caucasians
<i>n</i> (men)	17	17
Age (years)	28 ± 8	35 ± 8*
Height (cm)	171 ± 3	178 ± 7†
Body weight (kg)	96 ± 29	93 ± 24
Body fat (%)	29 ± 5	23 ± 7
Waist-to-thigh ratio	1.63 ± 0.11	1.63 ± 0.17
Fasting plasma glucose (mg/dl)	89 ± 5	87 ± 6
2-h plasma glucose (mmol/l)	103 ± 17	106 ± 23
Fasting plasma insulin (μU/ml)	27 ± 8	25 ± 8
Fasting PP (pg/ml)	79 ± 39	69 ± 30
Systolic blood pressure (mmHg)	124 ± 9	120 ± 10
Diastolic blood pressure (mmHg)	77 ± 6	75 ± 7
Heart rate (bpm)	63 ± 11	62 ± 7

Data are means ± SD. Symbols indicate significant differences between Pima Indians and Caucasians (unpaired *t* test): **P* < 0.05; †*P* < 0.01.

purpose, the liquid meal contained 20 mg/kg of acetaminophen, the appearance of which in the plasma was determined at subsequent time points (−60, 30, 60, and 120 min). Blood pressure, heart rate, and electrocardiogram were monitored continuously throughout the experiment and documented at the same time points as the blood samples, and they were collected to measure PP, insulin, and glucose (−60, −45, −30, −20, 0, 5, 10, 15, 20, 30, 45, 60, and 120 min). Plasma PP concentrations were determined by a radioimmunoassay (Linco, St. Charles, MO). The within and between errors in this assay were 7 and 10%, respectively. Immediately before, immediately after, and 30 min after injection of the priming dose (2 min) of saline or atropine, ocular responses were examined by a pupillometer (Iscan, Burlington, MA), which allowed us to measure the pupil area and the near point of accommodation.

Statistical analyses. Statistical analyses were performed using the software of the SAS Institute (Cary, NC). Results are given as means ± SD unless indicated otherwise. The values for plasma insulin were logarithmically transformed before analysis to approximate normal distributions.

Racial differences in autonomic nervous system tests were assessed by unpaired *t* test. ANOVA for repeated measures was used to assess the effect of race and intervention (to exclude possible carryover effects of consecutive atropine studies) on fasting plasma insulin, glucose, and PP concentrations at baseline (−60 to −30 min of the study) and during infusion of atropine (−30 to 0 min).

Areas under the curve (AUC) for early (AUC_{0–30 min}) and total (AUC_{0–120 min}) plasma insulin, glucose, PP, and acetaminophen were calculated using the trapezoid method. To account for differences in peripheral glucose uptake and any residual effect of gastric emptying rate not captured by the acetaminophen test and racial differences in percentage of body fat, all time points for insulin and glucose of all four atropine studies were used to adjust plasma insulin for plasma glucose. AUCs for early (AUC_{0–30 min}) and total (AUC_{0–120 min}) adjusted plasma insulin secretory responses were then calculated. ANOVA for repeated measures was used to assess the effects of race and intervention and the interaction between race × intervention on secretory responses for plasma insulin, glucose, PP, and acetaminophen. The race effect within each atropine study was assessed post hoc by unpaired *t* test. The effect of atropine on blood pressure, heart rate, pupillometry, and near point of accommodation was similarly assessed by ANOVA.

RESULTS

The anthropometric and metabolic characteristics of the study populations are summarized in Table 1. Pima Indians were younger and shorter and had a slightly higher body fat content than Caucasians (Table 1). There were no differences in heart rate in response to deep breathing (*P* = 0.9), prolongation of R-R interval in response to Valsalva maneuver (*P* = 0.6), or decrease of systolic blood pressure in response to standing up (*P* = 0.4) between

TABLE 2
Autonomic nervous system test results in 17 Pima Indians and 17 Caucasians

	Pima Indians	Caucasians
Change in the heart rate during deep breathing (bpm)	9 ± 4	9 ± 2
Ratio of maximum to minimum R-R interval	1.4 ± 0.1	1.4 ± 0.1
Change in systolic blood pressure between standing up and supine (mmHg)	−12 ± 7	−9 ± 8
Change in diastolic blood pressure between hand grip and resting (mmHg)	19 ± 10	9 ± 7*

*Significant differences between Pima Indians and Caucasians (unpaired *t* test), *P* < 0.05.

Pima Indians and Caucasians (Table 2). Diastolic blood pressure in response to the hand-grip test increased more in Pima Indians than in Caucasians (*P* = 0.04) (Table 2). There was no correlation between fasting PP and any of the measurement of cardiovascular autonomic nervous system activity (*r* = 0.31, *P* = 0.2 for the change in heart rate, *r* = 0.05, *P* = 0.8 for ratio of maximal and minimal R-R interval, *r* = −0.15, *P* = 0.5 for change in systolic blood pressure in response to standing up, and *r* = −0.18, *P* = 0.4 for change in diastolic blood pressure in response to hand-grip trip). There was no correlation between fasting insulin and any of the measurements of PNS activity (*r* = −0.05, *P* = 0.8 for the change in heart rate, and *r* = −0.31, *P* = 0.2 for ratio of maximal to minimal R-R interval).

Effect of PNS blockade on plasma insulin, glucose, and PP

Fasting plasma insulin, glucose, and PP. Mean plasma insulin, glucose, and PP during the baseline period before atropine administration (−60 to −30 min) did not differ between Pima Indians and Caucasians on any of the study days (all *P* > 0.3).

Fasting plasma insulin and PP concentrations decreased (both *P* < 0.0001 for intervention effect), whereas fasting plasma glucose concentration did not change with increasing dose of atropine (*P* > 0.5). Fasting plasma insulin, PP, and glucose concentrations during the atropine infusions but before consumption of the meal did not differ between Pima Indians and Caucasians (all *P* > 0.05, for race effect), and there were no differences in response to atropine between Pima Indians and Caucasians (*P* = 0.9 for insulin, *P* = 0.06 for PP, and *P* = 0.5 for glucose and for race × intervention effect).

Postprandial changes of plasma insulin, glucose, PP, and acetaminophen. The time course of the change of plasma PP, insulin, and glucose in response to the meal at all doses of atropine for both Pima Indians and Caucasians is shown in Fig. 2.

Plasma acetaminophen AUC (AUC_{0–120 min}) decreased with increasing atropine (*P* = 0.0001), but there was no difference in response to atropine administration between Pima Indians and Caucasians (*P* = 0.9) (Fig. 3A). As expected, overall there was a positive association between plasma acetaminophen and glucose concentrations (*r* = 0.56, *P* = 0.0001). Nevertheless, because plasma glucose

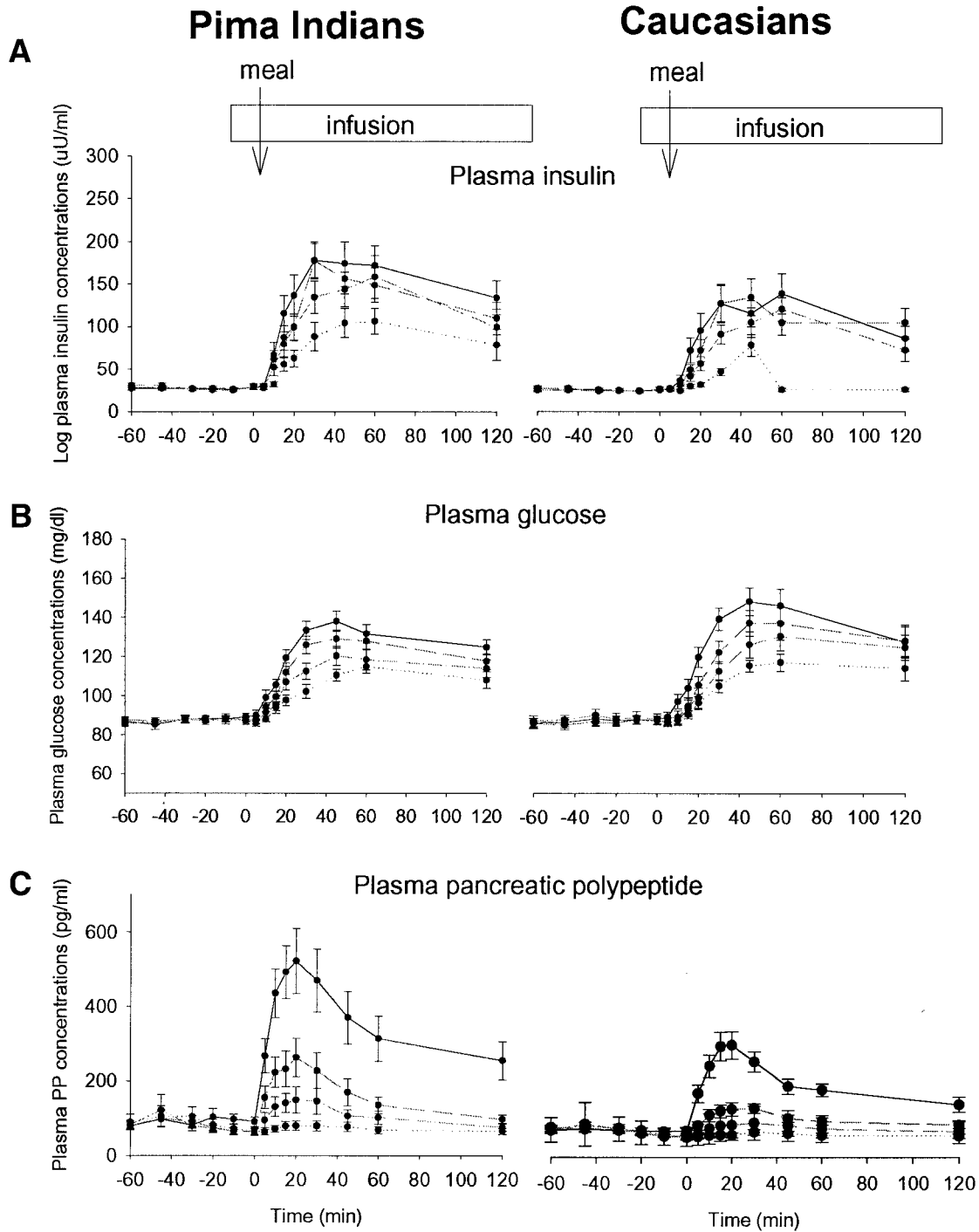


FIG. 2. Time course of plasma insulin (A), glucose (B), and PP (C) with SEs during the four interventions (atropine studies [ARs]), i.e., AR1 study (placebo, full line), AR2 (dose $2.5 \mu\text{g} \cdot \text{kg FFM}^{-1} \cdot \text{h}^{-1}$, dashed line), AR3 (dose $5 \mu\text{g} \cdot \text{kg FFM}^{-1} \cdot \text{h}^{-1}$, dotted dashed line), and AR4 (dose $10 \mu\text{g} \cdot \text{kg FFM}^{-1} \cdot \text{h}^{-1}$, dotted line).

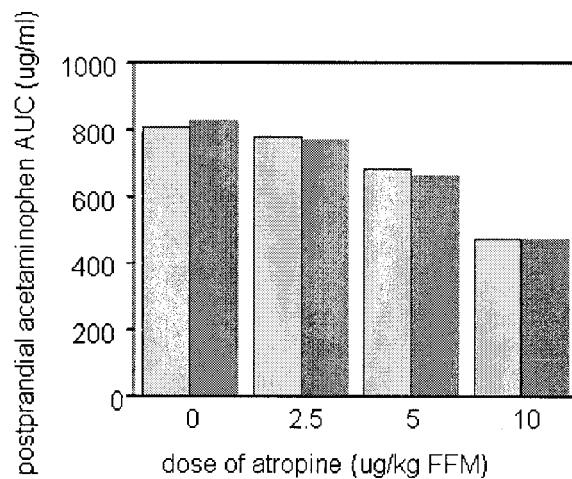
concentrations after a meal vary not only in relation to gastric emptying rate but also to the rate of peripheral tissue uptake (Fig. 2), plasma insulin responses were adjusted for plasma glucose levels to account for this effect (Fig. 3B).

Total postprandial PP secretion during the placebo infusion were 2.1-fold higher in Pima Indians than Caucasians ($P = 0.02$) and decreased with increasing doses of atropine ($P = 0.0001$). The decrease in total PP secretory responses was greater in Pima Indians than Caucasians

($P = 0.02$, for race \times intervention effect). Total postprandial insulin responses ($\text{AUC}_{0-120 \text{ min}}$) tended to be higher in Pima Indians compared with Caucasians (2.9-fold, $P = 0.06$) and decreased with increasing doses of atropine ($P = 0.001$), but there was no difference in overall response to atropine between Pima Indians and Caucasians ($P = 0.6$). The total postprandial glucose AUC was similar in Pima Indians and Caucasians ($P = 0.7$) and decreased with increasing atropine ($P = 0.001$), and there was no difference in response to atropine between Pima Indians

A

race effect=0.5 intervention effect=0.0001 race*intervention effect=0.9



B

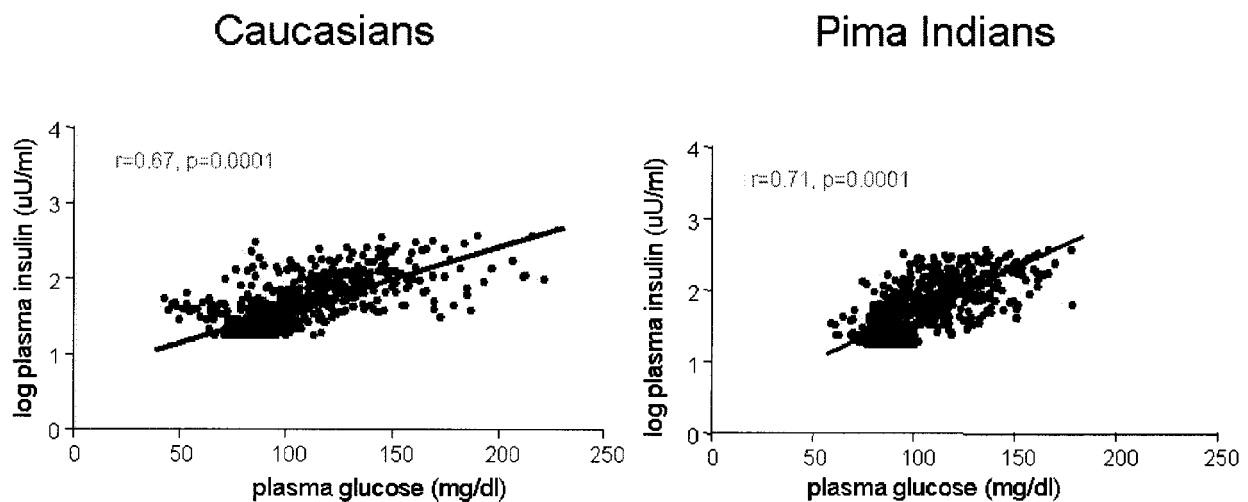


FIG. 3. A: The dose-response effect for total postprandial plasma acetaminophen areas under the curve ($AUC_{0-120 \text{ min}}$) during four interventions in Pima Indians and Caucasians. B: Relationship between log plasma insulin and glucose for all of the points and all four atropine studies for Pima Indians and Caucasians.

and Caucasians ($P = 0.5$, Fig. 2). The mean adjusted insulin AUC was larger in Pima Indians than in Caucasians ($P = 0.03$) and decreased with increasing atropine dose ($P = 0.01$), and there was no difference in response to atropine between Pima Indians and Caucasians ($P = 0.6$). There was no correlation between overall postprandial PP response and acetaminophen concentrations during the four interventions ($r = -0.05$, $P = 0.2$).

Early postprandial adjusted insulin and PP secretory responses ($AUC_{0-30 \text{ min}}$) were 2.5-fold and 2-fold higher in Pima Indians compared with Caucasians, respectively (Fig. 4). Secretion of adjusted insulin and PP was dose-dependently inhibited by atropine (Fig. 4). Increasing

doses of atropine attenuated the ethnic difference in PP but not in adjusted insulin responses (Fig. 4).

Effect of PNS blockade on blood pressure, heart rate, and pupillometry. Baseline systolic and diastolic blood pressure and heart rate during baseline did not differ between Pima Indians and Caucasians and the atropine studies (all $P > 0.4$) (Fig. 5).

Systolic and diastolic blood pressure during the atropine infusion increased with increasing dose of atropine (all $P < 0.001$ for intervention effect), but did not differ between Pima Indians and Caucasians (both $P > 0.2$), and there was no difference in response to atropine between Pima Indians and Caucasians (all $P > 0.2$).

Early postprandial (0-30 min)

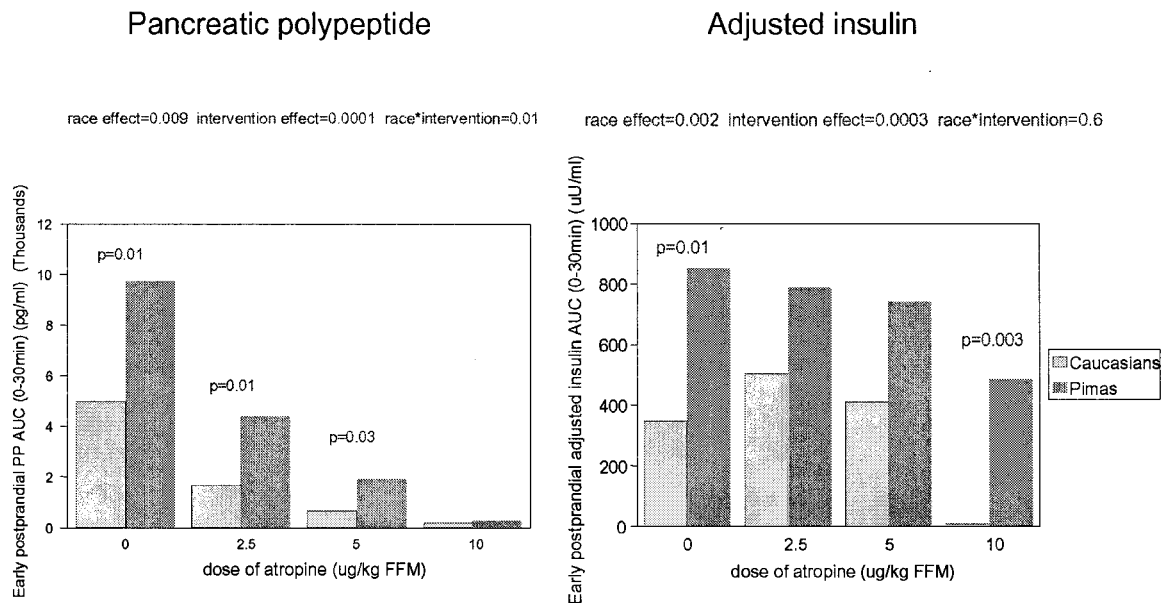


FIG. 4. The dose-response effect for early postprandial areas under the curve ($AUC_{0-30 \text{ min}}$) for plasma PP and adjusted insulin (for glucose and percentage of body fat) during four interventions in Pima Indians and Caucasians. ANOVA for repeated measures was used to assess the effect of race and intervention and the interaction between race \times intervention on secretory responses for early plasma insulin and PP. The race effect within each atropine study was assessed post hoc by unpaired *t* test.

Systolic and diastolic blood pressure AUCs ($AUC_{0-120 \text{ min}}$) in response to meal ingestion did not change with increasing doses of atropine and did not differ between Pima Indians and Caucasians, and there was no difference in response to atropine between Pima Indians and Caucasians ($P = 0.9$). Heart rate AUC in response to the meal ingestion ($AUC_{0-120 \text{ min}}$) increased with increasing dose of atropine ($P = 0.001$) and was higher in Pima Indians than in Caucasians ($P = 0.02$), and there was a greater response to atropine in Caucasians than in Pima Indians ($P = 0.04$). There was no effect of race or intervention on the pupil size or near point of accommodation (all $P > 0.1$).

DISCUSSION

We hypothesized that an increased PNS input to the pancreas contributes to hypersecretion of insulin and hyperinsulinemia of Pima Indians. We reasoned that if our hypothesis was correct then a graded infusion of the PNS blocker atropine should mitigate the ethnic differences in PP and in insulin secretion in response to meal ingestion. We found that PNS blockade abolished the ethnic difference in plasma PP but not in insulin secretory responses, suggesting that an increased PNS input of the β -cells is not the primary cause of hyperinsulinemia in Pima Indians.

What is the significance of hyperinsulinemia in Pima Indians? We have shown that the variability in fasting plasma insulin concentrations is only partially ($\sim 55\%$) explained by the difference in insulin sensitivity (2) and that "relative" hyperinsulinemia in Pima Indians with normal glucose tolerance is an independent predictor of type 2 diabetes, probably due to a deleterious effect on insulin secretion (5). Hyperinsulinemia is also an independent risk factor for cardiovascular disease (26) and may increase the risk of Alzheimer's disease (27).

Consistent with previous reports (14), we found that Pima Indians had larger postprandial plasma PP responses than Caucasians. As expected, increasing doses of atropine led to a dose-dependent reduction of the postprandial increase of PP. Finally, the highest dose of atropine completely abolished the postprandial increase of PP, an effect that was observed in previous studies (17–19,28–30) of other populations. Unlike previous reports, we did not observe higher fasting plasma PP levels in Pima Indians than in Caucasians. This may be due to the relatively small sample size in the present study. Interestingly, muscarinic blockade tended to produce a more marked dose-dependent decrease in fasting plasma PP levels in Pima Indians than in Caucasians.

Hyperinsulinemia in Pima Indians is most pronounced during the first 30 min of a meal (2). In the present study, we showed higher early postprandial insulin secretion and a trend for higher total postprandial insulin secretion in Pima Indians compared with Caucasians. Both early postprandial insulin and total postprandial insulin secretion were inhibited by increasing doses of atropine. The significant contribution of the PNS to insulin secretion in response to meal ingestion has been demonstrated in both animals (28,30) and humans (29,31). Despite the ethnic difference, insulin secretion, and the decrease in plasma insulin response with increasing doses of atropine, there was no ethnic difference in the ability of cholinergic blockade to reduce postprandial insulin secretion. Thus, while the lowest dose of atropine normalized the PP response in Pima Indians to the magnitude measured during saline administration in Caucasians, this same dose did not appreciably reduce the difference in insulin secretion between Pima Indians and Caucasians. Furthermore, with PP completely suppressed at the highest dose of

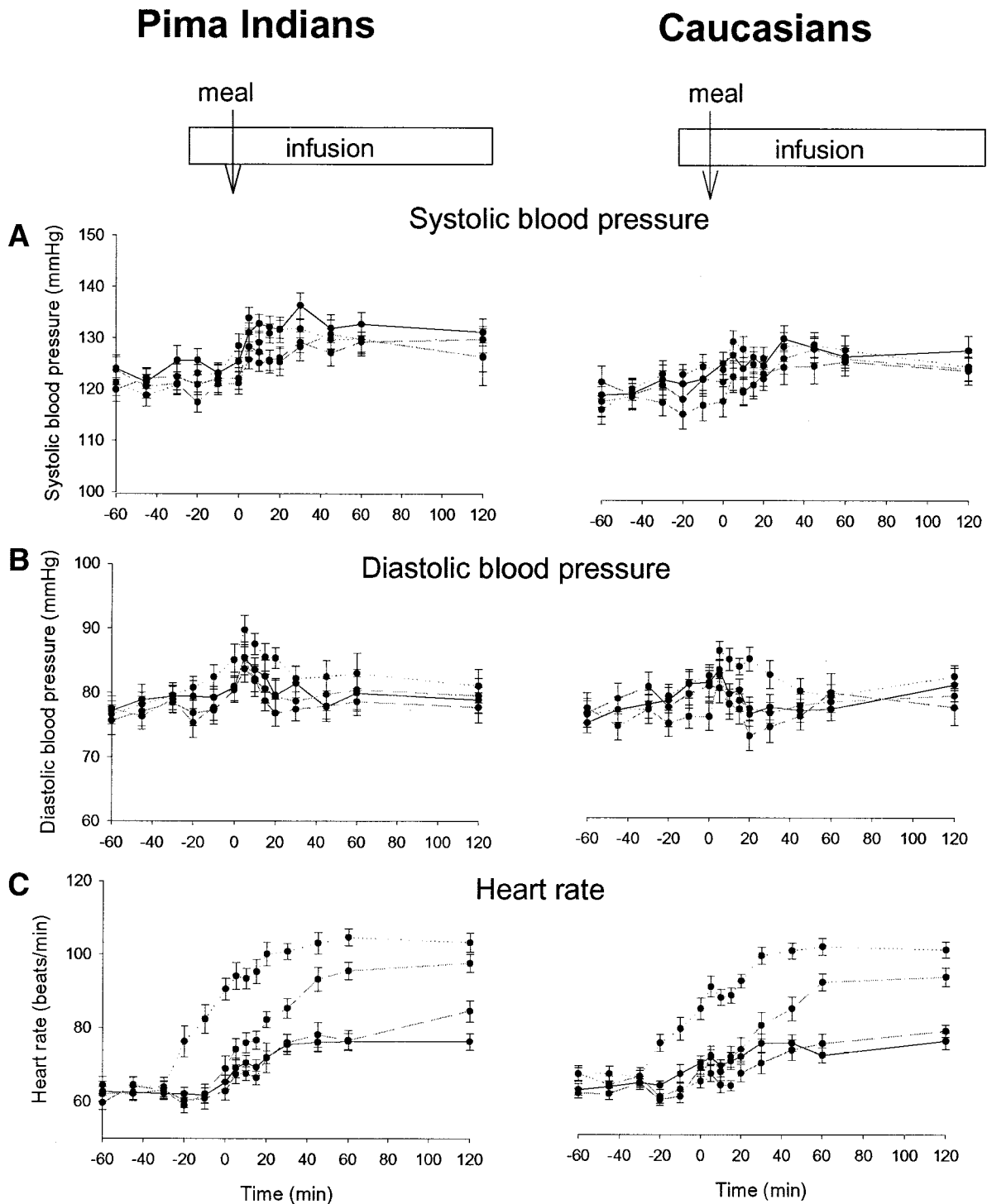


FIG. 5. Time course of systolic and diastolic blood pressure and heart rate with SEs during the four interventions (atropine studies [AR]), i.e., AR1 study (placebo, full line), AR2 (dose $2.5 \mu\text{g} \cdot \text{kg FFM}^{-1} \cdot \text{h}^{-1}$, dashed line), AR3 (dose $5 \mu\text{g} \cdot \text{kg FFM}^{-1} \cdot \text{h}^{-1}$, dotted dashed line), and AR4 (dose $10 \mu\text{g} \cdot \text{kg FFM}^{-1} \cdot \text{h}^{-1}$, dotted line).

atropine, insulin secretion was still present and was still significantly higher in the Pima Indians than in Caucasians. Taken together, these data suggest that PNS contributes to an increase of plasma insulin secretion in response to a meal; however, the enhanced insulin secretion in adult Pima Indians is not due to an exaggerated PNS stimulation of the β -cells.

There are some limitations to our study. First, we did not achieve a complete blockade of PNS activation by

atropine, which antagonizes only cholinergic muscarinic receptors but not the release or actions of other PNS-signaling neuropeptides (26). However, it was shown that nicotinic blockade provides only a further 9% decrease in insulin secretion compared with muscarinic blockade (28). A second limitation is that we did not have a more accurate measure of insulin sensitivity (i.e., glucose uptake measured by euglycemic clamp) available in our study than glucose and percentage of body fat to adjust

plasma insulin and thereby account for ethnic differences in insulin sensitivity. It could be argued that Pima Indians did not decrease their postprandial plasma insulin more than Caucasians because they are more insulin resistant. Finally, we do not have estimates of insulin clearance in this study. However, in previous studies insulin clearance was not found to be different in Pima Indians compared with Caucasians (2) and the effect of atropine on C-peptide paralleled the effects on insulin, suggesting that insulin secretion and insulin clearance are not differentially affected by PNS blockade (29).

There are data to suggest that PNS input contributes directly to enhanced insulin secretion of obesity in young animals (obese Zucker rats), but that in older animals with established obesity the primary contributor to hyperinsulinemia is increased β -cell mass (10). This suggests the possibility of a hyperstimulation of the pancreas by the PNS chronically leading to an increase in β -cell mass that may no longer be acutely affected by pharmacological blockade once established. To our knowledge, no studies have addressed this possibility.

If an increased PNS drive to the pancreas does not explain the hyperinsulinemia of Pima Indians then the question arises of what other hormones might contribute to this ethnic difference. In this respect, it should be pointed out that incretins (glucagon-like peptide 1 and gastric inhibitory peptide) and catecholamines play an important role in the modulation of postprandial insulin secretion (28–31). Other possible etiologic factors contributing to hyperinsulinemia in Pima Indians may include downregulation of insulin receptors in pancreatic β -cells leading to impaired glucose sensing, plasma free fatty acid concentrations, β -cell insulin receptor expression, metabolic clearance rate of insulin, and abnormalities in central (hypothalamic) regulatory pathways (5). However, the contribution of these mechanisms was not addressed in this study.

Despite an ethnic difference in the PNS input to the pancreatic F-cells, there was no ethnic difference in measures of PNS activity to other organs (e.g., stomach, eye, or cardiovascular system). This suggests that PNS activity is not globally upregulated in Pima Indians compared with Caucasians. It is well established that PNS outflow is selective to different tissues and that the input to PNS-innervated organs is discretely activated under differing physiological conditions (32). Therefore, it is not too surprising that activation of the parasympathetic input to pancreatic F-cells can be exaggerated in response to meal ingestion in Pima Indians compared with Caucasians but that PNS activity to other organs or under other conditions is not exaggerated. Furthermore, the results of this study indicating increased cholinergic stimulation to PP-secreting F-cells but not to B-cells in Pima Indians provides evidence that there are likely additional differences in the PNS input to different cell types within an individual organ. Whether this cell-specific difference within the islet is anatomical (i.e., more parasympathetic nerve fibers adjacent to F-cells than β -cells), or functional, with enhanced F-cell sensitivity to acetylcholine, is not known.

In conclusion, results from this study confirm that compared with Caucasians, Pima Indians have an exaggerated PNS drive to the pancreas and more specifically to the

pancreatic F-cells. Thus, the hyperinsulinemia of this population does not appear to be due to increased vagal input to pancreatic β -cells.

ACKNOWLEDGMENTS

The authors thank Drs. K. Teff, R. Gingerich, and B. Jeanrenaud for stimulating discussions and Drs. Robert Hanson and Sayuko Kobes for help with statistical analyses. The nurses of the Clinical Research Unit and the staff of the metabolic kitchen are also acknowledged for their care of the patients in these studies.

REFERENCES

- Knowler WC, Pettitt DJ, Savage PJ, Bennett PH: Diabetes incidence in Pima Indians: contributions of obesity and parental diabetes. *Am J Epidemiol* 113:144–156, 1981
- Lillioja S, Nyomba BL, Saad MF, Ferraro R, Castillo C, Bennett PH, Bogardus C: Exaggerated early insulin release and insulin resistance in a diabetes-prone population: a metabolic comparison of Pima Indians and Caucasians. *J Clin Endocrinol Metab* 73:866–876, 1991
- Lillioja S, Mott DM, Spraul M, Ferraro R, Foley JE, Ravussin E, Knowler WC, Bennett PH, Bogardus C: Insulin resistance and insulin secretory dysfunction as precursors of non-insulin-dependent diabetes mellitus: prospective studies of Pima Indians. *N Engl J Med* 329:1988–1992, 1993
- Saad MF, Knowler WC, Pettitt DJ, Nelson RG, Mott DM, Bennett PH: Sequential changes in serum insulin concentration during development of non-insulin-dependent diabetes. *Lancet* 1:1356–1359, 1989
- Weyer C, Hanson R, Tataranni PA, Bogardus C, Pratley RE: A high fasting plasma insulin concentration predicts type 2 diabetes independent of insulin resistance: evidence for a pathogenic role of relative hyperinsulinemia. *Diabetes* 49:2094–2101, 2000
- Charles MA, Fontbonne A, Thibault N, Warnet JM, Rosselin GE, Eschwege E: Risk factors for NIDDM in white population: Paris Prospective Study. *Diabetes* 40:796–799, 1991
- Lundgren H, Bengtsson C, Blohme G, Lapidus L, Waldenstrom J: Fasting serum insulin concentration and early insulin response as risk determinants for developing diabetes. *Diabet Med* 7:407–413, 1990
- Pettitt DJ, Moll PP, Knowler WC, Mott DM, Nelson RG, Saad MF, Bennett PH, Kottke BA: Insulinemia in children at low and high risk of NIDDM. *Diabetes Care* 16:608–615, 1993
- Fukudo S, Virnelli S, Kuhn CM, Cochrane C, Feinglos MN, Surwit RS: Muscarinic stimulation and antagonism and glucoregulation in nondiabetic and obese hyperglycemic mice. *Diabetes* 38:1433–1438, 1989
- Fletcher JM, McKenzie N: The parasympathetic nervous system and glucocorticoid-mediated hyperinsulinaemia in the genetically obese (fa/fa) Zucker rat. *J Endocrinol* 118:87–92, 1988
- Rohner-Jeanrenaud F, Ionescu E, Jeanrenaud B: The origins and role of efferent vagal nuclei in hyperinsulinemia in hypothalamic and genetically obese rodents. *J Auton Nerv Syst* 9:173–184, 1983
- Sainsbury A, Rohner-Jeanrenaud F, Cusin I, Zakrzewska KE, Halban PA, Gaillard RC, Jeanrenaud B: Chronic central neuro-peptide Y infusion in normal rats: status of the hypothalamo-pituitary-adrenal axis, and vagal mediation of hyperinsulinaemia. *Diabetologia* 40:1269–1277, 1997
- Berthoud H, Jeanrenaud B: Acute hyperinsulinemia and its reversal by vagotomy after lesions of the ventromedial hypothalamus in anaesthetized rats. *Endocrinology* 105:146–151, 1979
- Gingerich RL, Nagulesparan M, Bennion L, Dye ES, Bauman WA: Pancreatic polypeptide in Pima Indians: the influence of obesity and diabetes. *Metabolism* 34:25–29, 1985
- Weyer C, Salbe AD, Lindsay RS, Pratley RE, Bogardus C, Tataranni PA: Exaggerated pancreatic polypeptide secretion in Pima Indians: can an increased parasympathetic drive to the pancreas contribute to hyperinsulinemia, obesity, and diabetes in humans? *Metabolism* 50:223–230, 2001
- Schwartz TW, Holst JJ, Fahrenkrug J, Jensen SL, Nielsen OV, Rehfeld JF, de Muckadell OB, Stadil F: Vagal, cholinergic regulation of pancreatic polypeptide secretion. *J Clin Invest* 61:781–789, 1978
- Schwartz TW, Rehfeld JF, Stadil F, Larson LI, Chance RE, Moon N: Pancreatic-polypeptide response to food in duodenal-ulcer patients before and after vagotomy. *Lancet* 1:1102–1105, 1976
- Feldman M, Richardson CT, Taylor IL, Walsh JH: Effect of atropine on vagal release of gastrin and pancreatic polypeptide. *J Clin Invest* 63:294–298, 1979
- Tappy L, Chioloro R, Randin JP, Burckhardt P, Felber JP: Effects of

- cholinergic stimulation and antagonism on plasma insulin concentration in lean and obese human subjects. *Horm Metab Res* 18:821–826, 1986
20. Del Rio G, Procopio M, Bondi M, Marrama P, Menozzi R, Oleandri SE, Grottoli S, Maccario M, Velardo A, Ghigo E: Cholinergic enhancement by pyridostigmine increases the insulin response to glucose load in obese patients but not in normal subjects. *Int J Obes Relat Metab Disord* 21:1111–1114, 1997
 21. World Health Organization: *Diabetes Mellitus: Report of a WHO Study Group*. Geneva, World Health Org., 1985 (Tech. Rep. Ser. no. 17)
 22. Tataranni PA, Ravussin E: Use of dual-energy X-ray absorptiometry in obese individuals. *Am J Clin Nutr* 62:730–734, 1995
 23. Rossi M, Marti G, Ricordi L, Fornasari G, Finardi G, Fratino P, Bernardi L: Cardiac autonomic dysfunction in obese subjects. *Clin Sci (Lond)* 76:567–572, 1989
 24. Medhus AW, Lofthus CM, Bredeesen J, Husebye E: Gastric emptying: the validity of the paracetamol absorption test adjusted for individual pharmacokinetics. *Neurogastroenterol Motil* 13:179–185, 2001
 25. Medhus AW, Sandstad O, Bredeesen J, Husebye E: Delay of gastric emptying by duodenal intubation: sensitive measurement of gastric emptying by the paracetamol absorption test. *Aliment Pharmacol Ther* 13:609–620, 1999
 26. Festa A, D'Agostino RJ, Mykkanen L, Tracy RP, Zaccaro D, Hales C: Relative contribution of insulin and its precursors to fibrinogen and PAI-1 in a large population with different states of glucose tolerance: the Insulin Resistance Atherosclerosis Study (IRAS). *Arterioscler ThrombVasc Biol* 19:562–568, 1999
 27. Watson GS, Peskind ER, Asthana S, Puganan K, Wait C, Chapman D, Schwartz MW, Plymate S, Craft S: Insulin increases CSF Abeta42 levels in normal older adults. *Neurology* 60:1899–1903, 2003
 28. D'Alessio DA, Kieffer TJ, Taborsky GJ Jr, Havel PJ: Activation of the parasympathetic nervous system is necessary for normal meal-induced insulin secretion in rhesus macaques. *J Clin Endocrinol Metab* 86:1253–1259, 2001
 29. Teff KL, Townsend RR: Early phase insulin infusion and muscarinic blockade in obese and lean subjects. *Am J Physiol* 277:R198–R208, 1999
 30. Ahren B, Holst JJ: The cephalic insulin response to meal ingestion in humans is dependent on both cholinergic and noncholinergic mechanisms and is important for postprandial glycemia. *Diabetes* 50:1030–1038, 2001
 31. Bentham L, Munding TO, Taborsky GJ Jr: Meal-induced insulin secretion in dogs is mediated by both branches of the autonomic nervous system. *Am J Physiol Endocrinol Metab* 278:E603–E610, 2000
 32. Kelly J: Principles of the functional and anatomical organisation of the nervous system. In *Principles of Neural Science*. Kandel ER, Schwartz JH, Eds. San Diego, CA, Elsevier Press, 1985, p. 211–221