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Contribution of nonesterified fatty acids to insulin resistance in the elderly with normal fasting but diabetic 2-hour postchallenge plasma glucose levels: the Baltimore Longitudinal Study of Aging

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Abstract

Isolated postchallenge hyperglycemia (IPH) with normal fasting plasma glucose <100 mg/dL and plasma glucose with diabetic 2-hour plasma glucose ≥200 mg/dL after an oral glucose tolerance test (OGTT) is a common occurrence in the elderly. We sought to understand what unique characteristics this population might have that puts it at risk for this particular metabolic finding. We therefore conducted a longitudinal study of volunteers in the Baltimore Longitudinal Study of Aging (BLSA). All volunteers had an OGTT performed (75 g) on 2 or more occasions. We measured plasma levels of glucose, insulin, C-peptide, glucagon-like peptide-1 (GLP-1), glucose-dependent insulinotropic peptide (GIP), ghrelin, leptin, adiponectin, resistin, C-reactive protein, cytokines, and their soluble receptors, as well as nonesterified free fatty acids (NEFAs). We determined that 22 subjects in BLSA had IPH, accounting for 2.1% of the BLSA population. All 22 were older than 65 years. They were then matched by age, sex, and body mass index to 12 subjects who had isolated impaired glucose tolerance (IGT) and 15 subjects with normal glucose tolerance (NGT). All subjects had normal fasting glucose levels <100 mg/dL in accordance with the American Diabetes Association Expert Committee on the Classification and Diagnosis of Diabetes Mellitus criteria (2003). We found that subjects with IPH had similar plasma insulin levels to the other 2 groups, except at the 2-hour time when their insulin levels were higher than NGT ($P < .05$). Although there was a clear trend for differences in the insulinogenic index, the areas under the curves for insulin, systolic blood pressure, adiponectin, and C-reactive protein across the glucose tolerance categories revealed no statistical significance. Cytokines and their soluble receptors, gut hormones, and adipokines were similar in all 3 groups. The NEFA levels were significantly elevated in the fasting state ($P < .05$) in the IPH compared with NGT, with IGT intermediate between the other 2 groups. The rate of clearance of NEFAs after the OGTT decreased progressively from the NGT to the IPH group (in micromoles per liter per minute: NGT, 11.9 vs IGT, 7.6 vs IPH, 3.0). We conclude that the rate of suppression of lipolysis in the elderly determines the sensitivity of glucose uptake to insulin after OGTT.

1. Introduction

Diabetes is characterized biochemically by a fasting plasma glucose (FPG) level ≥126 mg/dL, a plasma glucose ≥200 mg/dL 2 hours after an oral glucose tolerance test (OGTT), or both [1]. The number of subjects with isolated postchallenge hyperglycemia (IPH), defined by FPG <126 mg/dL and 2-hour plasma glucose ≥200 mg/dL, was estimated at 9.8 % in a national sample of US adults with type 2 diabetes mellitus (age, 40-74 years) [2]. The prevalence of IPH in the Tehran Lipid and Glucose Study of nondiabetic subjects aged >20 years was 2.5% using an FPG of <126 mg/dL; but using an FPG of <100 mg/dL, the prevalence was only 0.54% [3]. The Diabetes Epidemiology: Collaborative Analysis of Diagnostic Criteria in Europe study concluded that one third of the older subjects (60-79 years) with FPG <126 mg/dL also had IPH and, most importantly, that subjects with IPH had an elevated risk of mortality that was similar to that of subjects with FPG ≥126 mg/dL [4]. Several other studies concur with the Diabetes

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Epidemiology: Collaborative Analysis of Diagnostic Criteria in Europe study in estimating that IPH places people at risk for cardiovascular disease and mortality [5-7]. Moreover, previous results from the Baltimore Longitudinal Study of Aging (BLSA) determined that impaired glucose tolerance (IGT), but not impaired fasting glucose (IFG), is associated with increased levels of coronary heart disease risk factors [8]. It is evident therefore that prior studies defining IPH include subjects with a wide range of FPG from normal to impaired, thus introducing a separate variable. To remove any influence of IFG, we restricted our IPH definition to only subjects with normal fasting glucose (NFG)/diabetic glucose tolerance (DGT), defining NFG as plasma glucose levels of <100 mg/dL, based on the American Diabetes Association classification of 2003 [1]. The prevalence of diabetes and glucose intolerance, of course, increases with age; and the pathogenesis of carbohydrate intolerance in the elderly has been an area of active research. Among 534 subjects (26-92 years old) with NFG in BLSA, we found 22 subjects with IPH, all older than 65 years. On the other hand, we were unable to find a single subject younger than 65 years in the IPH group. From our data, it therefore seems that IPH with FPG <100 mg/dL is unique to the elderly population. Although IPH patients comprise only 2.1% of the total number of subjects in the BLSA, they nevertheless provide valuable insights for studying mechanisms of glucose intolerance as a function of age. With an average body mass index (BMI) of 26.4 ± 1.0 kg/m^2 and an average age of 81.1 ± 2.2 years, this group has morphological traits more similar to nondiabetic subjects than to subjects with diabetic fasting glucose (DFG) levels and DGT.

In this present study, we sought to characterize and understand the pathophysiology of IPH and compare and contrast it with the 10.9% of BLSA subjects with IGT and with the 56.9% of BLSA subjects having normal glucose tolerance (NGT). The NFG depends on adequate basal insulin secretion and insulin sensitivity, mainly in the liver, to control hepatic glucose output. Abnormalities of these 2 requirements have been documented to be characteristic of IFG and DFG levels [9]. On the other hand, impaired and diabetic 2-hour plasma glucose levels after an OGTT are thought to reflect peripheral insulin resistance [10]. We found that the IPH subjects had significantly elevated nonesterified free fatty acid (NEFA) levels that were not suppressed during the early phase of insulin secretion. In addition, subjects with IPH are among the oldest subjects in the BLSA.

2. Methods

2.1. Selection of subjects

The BLSA has been in existence since 1958 to study normal aging and evaluate biomarkers of age-associated diseases in the greater Baltimore, Washington, DC, area [11]. This study was approved by the Committee on Human Investigation of the Medstar Research Institute. All volunteers were informed about the nature of the study and provided written informed consent, in accordance with the Helsinki II declaration. Our diabetes section has participated in the BLSA since April 2001, performing modified OGTT and quantifying plasma hormone levels. For the modified OGTT, fasting plasma was collected at baseline (0 minute), after which subjects drank 75 g glucose in 300 mL solution (SunDex; Fisherbrand, Pittsburgh, PA) and blood samples were drawn at 5, 10, 15, 20, 40, 60, 80, 100, and 120 minutes (2 hours) after oral administration [12,13]. Using the results of plasma glucose concentrations at 0 minute and 2 hours, we classified every BLSA subject into one of 9 different groups, as shown in Table 1. Subjects with NFG (≤99 mg/dL) and normal 2-hour glucose tolerance (NGT, ≤139 mg/dL) belong to group 1. Subjects with NFG and impaired 2-hour glucose tolerance (IGT, 140-199 mg/dL), IFG (100-125 mg/dL) and IGT, and IFG/NGT belong to groups 2, 3, and 4, respectively. Subjects with NFG and diabetic 2-hour glucose tolerance (DGT, ≥200 mg/dL), IFG/DGT, DFG (≥126 mg/dL)/DGT, DFG/IGT, and DFG/NGT are classified in groups 5, 6, 7, 8, and 9, respectively. There are no subjects presently in group 9. A thousand OGTTs were performed between April 2001 and September 2005, of which 534 had NFG. Among this group, only 22 subjects belonged to group 5 (IPH), of which only 15 were first diagnosed as IPH. They were studied on at least 2 occasions: their age range was 66 to 91 years, and BMI range was 20 to 30 kg/m^2. Thirty-six subjects had IGT on at least 2 occasions. When subjects had multiple visits and where the results of OGTTs were similar, results from the last visit were used. We excluded subjects with hemolyzed plasma samples, those with missing plasma samples at 3 or more time points, and

Table 1
Classification of glucose tolerance

2-h plasma post OGTT	≥200 mg/dL (11.1 mmol/L)	NFG/DGT (group 5)	IFG/DGT (group 6)	DFG/DGT (group 7)
	140-199 mg/dL (7.78-11.06 mmol/L)	NFG/IGT (group 2)	IFG/IGT (group 3)	DFG/IGT (group 8)
	≤139 mg/dL (7.7 mmol/L)	NFG/NGT (group 1)	IFG/NGT (group 4)	DFG/DGT (group 9)
		≤99 mg/dL (5.5 mmol/L)	100-125 mg/dL (5.6-6.9 mmol/L)	≥126 mg/dL (7.0 mmol/L)
		FPG levels		

Subjects with NFG (≤99 mg/dL) and NGT (≤139 mg/dL) belong to group 1. Subjects with NFG and IGT (140-199 mg/dL), IFG (100-125 mg/dL) and IGT, and IFG/NGT belong to groups 2, 3, and 4, respectively. Subjects with NFG and DGT (≥200 mg/dL), IFG/DGT, DFG (≥126 mg/dL)/DGT, DFG/IGT, and DFG/NGT are classified in groups 5, 6, 7, 8, and 9, respectively.

Table 2
Characteristics of all the subjects

	Group 1 (NGT) (n = 15)	Group 2 (IGT) (n = 12)	Group 5 (IPH) (n = 11)
Age (y)	78.0 ± 2.2	78.7 ± 2.0	81.1 ± 2.2
Sex (F/M)	6:9	4:8	5:6
BMI (kg/m^2)	26.2 ± 0.6	25.5 ± 0.7	26.4 ± 1.0
HbA_{1C} (%)	5.6 ± 0.2	5.8 ± 0.2	5.9 ± 0.2
24-h microalbumin (mg)	7.5 ± 5.3	10.5 ± 9.0	18.4 ± 22.5
Total cholesterol (mg/dL)	205.9 ± 16.7	189.4 ± 11.0	179.3 ± 10.0
LDL (mg/dL)	117.2 ± 10.3	114.1 ± 8.7	106.3 ± 9.1
HDL (mg/dL)	69.1 ± 7.6	56.1 ± 3.4	51.8 ± 5.4
Triglycerides (mg/dL)	84.0 ± 11.6	92.2 ± 12.7	106.0 ± 14.1
% fat (trunk)	15.6 ± 1.4	13.4 ± 3.6	20.6 ± 1.0
% fat (total body)	31.2 ± 3.6	30.7 ± 2.8	35.3 ± 2.0
% lean (total body)	64.0 ± 3.7	64.5 ± 3.1	61.9 ± 1.8
Systolic BP (%, BP ≥130 mm Hg)	131.0 ± 5.1 (62.5)	137.8 ± 3.8 (66.7)	142.7 ± 4.7 (72.7)
Diastolic BP (%, BP ≥80 mm Hg)	68.7 ± 2.2 (20)	71.2 ± 2.6 (20)	68.5 ± 5.2 (18)
Fasting (0 min)			
C-Peptide (pmol/L)	0.4 ± 0.1	0.7 ± 0.2	0.7 ± 0.1
GLP-1 (pmol/L)	10.1 ± 4.1	3.6 ± 0.6	14.3 ± 7.1
GIP (pg/mL)	13.3 ± 4.2	9.2 ± 0.8	8.2 ± 1.2
Adiponectin (mg/L)	13.7 ± 2.0	12.2 ± 2.2	8.4 ± 1.5
Ghrelin (pg/mL)	416.4 ± 60.8	317.1 ± 39.9	372.1 ± 96.4
Resistin (ng/mL)	7.9 ± 0.6	9.3 ± 1.1	8.3 ± 1.3
Leptin (ng/mL)	31.0 ± 6.9	22.1 ± 6.1	22.5 ± 6.0

Hemoglobin A_{1C} is expressed in means ± SD. All other values are expressed in means ± SEM. LDL indicates low-density lipoprotein; HDL, high-density lipoprotein.

those who had taken steroids (ie, prednisone) within 3 months of the OGTT and/or those who were on any glucose-lowering agents. Finally, there remained 11 subjects in group 5 (IPH) that we then matched for age, sex, and BMI with 12 subjects in group 2 (IGT) and 15 subjects in group 1 (NGT).

2.2. Plasma hormone and biochemical assays

We quantified plasma glucose concentration levels using a glucose analyzer (Beckman Instruments, Brea, CA). We measured hemoglobin A_{1c} (HbA_{1C}) with an automated DiaSTAT analyzer (Bio-Rad Laboratories, Hercules, CA). Plasma lipid levels were determined in the Clinical Core Laboratory (Research Resources Branch, National Institute on Aging [NIA]/National Institutes of Health) using an Auto Analyzer (Synchron CX-5, Beckman Instruments). Anthropometric measurements, including weight, height, and blood pressure (BP), were recorded at the time of the visit, as previously described in BLSA [14]. Percentage of total body and truncal fat mass and percentage of total lean body mass were estimated. We measured 24-hour urinary microalbumin levels with an Array 360 System (Beckman Instruments). We assayed plasma samples for insulin, C-peptide, and resistin by enzyme-linked immunosorbent assay (ELISA) (Alpco Diagnostics, Salem, NH) with intraassay variations of 4.8% to 9.0%, 2.9% to 4.8%, and 2.8% to 3.4% and interassay variations of 2.6% to 3.6%, 0.6% to 4.8%, and 5.1% to 6.9%, respectively. We also assayed plasma samples for leptin, glucagon-like peptide-1 (GLP-1), and glucose-dependent insulinotropic peptide (GIP) using ELISA (LINCO Research, St Charles, MO) with intraassay variations of 1.09% to 4.98%, 6.0% to 9.0%, and 3.0% to 8.8%, in addition to interassay variations of 3.89% to 5.33%, 7.0% to 13.0%, and 1.8% to 6.1%, respectively. We detected plasma interleukin (IL) 6sR, tumor necrosis factor (TNF) αsRI, and TNF-αsRII with ELISA (R and D Systems, Minneapolis, MN) possessing intraassay variations of 2.3% to 8.6%, 3.6% to 5.0%, and 3.6% to 5.0% and interassay variations of 6.5% to 9.6%, 3.7% to 8.8%, and 3.7% to 8.8%, respectively. We

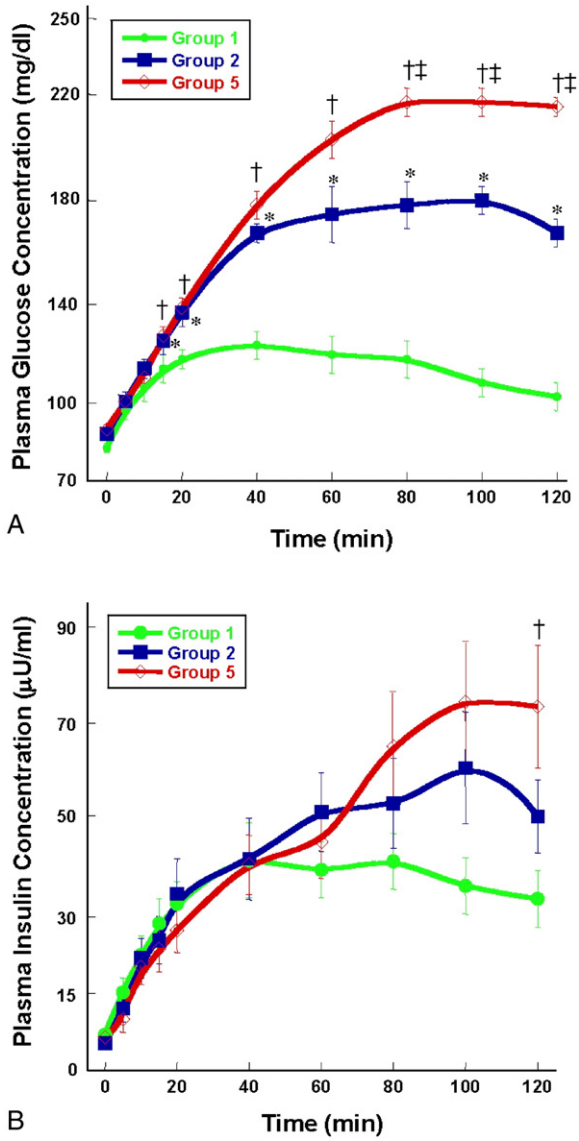

Fig. 1. Plasma glucose and insulin after administration of 75-g oral glucose (OGTT) to BLSA participants. A and B, Glucose and insulin levels in groups 1, 2, and 5 (Table 1) subjects. *Group 2 statistically significant compared with group 1 ($P < .05$), † group 5 statistically significant compared with group 1 ($P < .05$), ‡ group 5 statistically significant compared with group 2 ($P < .05$).

Table 3
Cytokine and cytokine soluble receptors

	Group 1 (NGT) (n = 15)	Group 2 (IGT) (n = 12)	Group 5 (IPH) (n = 11)
Fasting (0 min)			
TNF-αsRI (pg/mL)	125.0 ± 10.2	117.7 ± 8.9	175.5 ± 32.3
TNF-αsRII (pg/mL)	245.0 ± 16.8	247.5 ± 19.9	320.2 ± 41.8
IL-6sR (pg/mL)	358.5 ± 24.7	343.7 ± 24.0	337.4 ± 36.1
IL-6 (pg/mL)	36.0 ± 17.4	13.4 ± 7.2	25.2 ± 8.0
IL-12 (pg/mL)	1.0 ± 0.3	2.8 ± 1.9	15.9 ± 6.8* **
TNF-α (pg/mL)	5.5 ± 0.8	5.1 ± 1.0	6.2 ± 1.2
C-Reactive protein (ng/mL)	876.2 ± 158.4	1891.5 ± 709.5	2466.0 ± 1264.1

All values are expressed in means ± SEM.
* Group 5 statistically significant compared with group 1 ($P < .05$).

measured fasting plasma C-reactive protein levels by ELISA (Alpha Diagnostic International, San Antonio, TX) with an intraassay variation of 2.1% to 4.5% and an interassay variation of 3.0% to 7.0%. The human cytokine chemokine panel (LINCO Research) made it possible for us to customize an assay; and results for IL-6 and TNF-α were read using a Bio-Plex machine (Bio-Rad Laboratories) with an intraassay precision of 8.6% and 9.0% and an interassay precision of 12.7% and 10.9%, respectively. All cytokines and cytokine receptor assays were measured on the same day. We measured fasting plasma samples for adiponectin by radioimmunoassay (LINCO Research) with intraassay and interassay variations of 1.78% to 6.21% and 6.9% to 9.25%, respectively. In addition, we assayed plasma samples for total ghrelin using radioimmunoassay (Phoenix Pharmaceuticals, Belmont, CA) with calculated intraassay and interassay variations of 6.7% and 7.8%, respectively. Finally, we measured NEFAs in plasma using an enzymatic end point assay (WAKO Chemicals, Richmond, VA) with a precision variable range from 1.1% to 2.7%.

2.3. Statistical analysis

All values are shown as means ± SEM. We compared the sex and race ratios of all groups with the χ^2 test. We used a 1-way analysis of variance test and SAS (version 9.1; SAS Institute, Cary, NC) to compare body composition, hormone, BP, and NEFA levels and modeled for insulin sensitivity and insulin secretion, adjusting for age and BMI among all the groups. We quantified insulin sensitivity by calculating the homeostatic model assessment of insulin resistance (HOMA-IR) using FPG and insulin levels [15] and assessed early-phase insulin secretion by measuring the insulinogenic index ($II_{0\text{-}20\ minutes}$) as the ratio of the increment in the plasma insulin level to that of the plasma glucose level during the first 20 minutes after a modified OGTT [16]. We also calculated the insulin sensitivity index (ISI), metabolic clearance rates (MCRs), β-cell function during first-phase secretion (β-cell function, first phase) and second-phase secretion (β-cell function, second phase) [17-19], as well as oral glucose insulin sensitivity (OGIS: 0, 90 [mean of the 80- and 100-minute value], and 120) [20].

3. Results

3.1. Characteristics of subjects

The characteristics of all the subjects are presented in Table 2. These subjects had similar age (66-92 years) and BMI (22-30 kg/m^2) ranges and fasting glucose levels

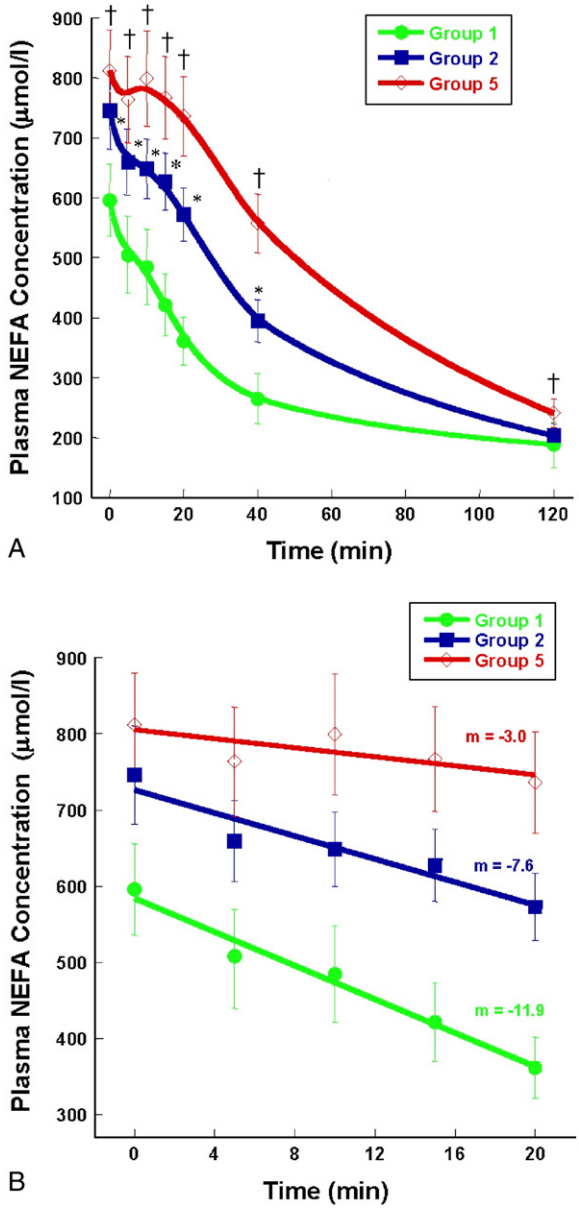

Fig. 2. Plasma NEFAs after administration of 75-g oral glucose (OGTT) to BLSA participants. A, The NEFA levels in groups 1, 2, and 5 (Table 1) subjects. * Group 2 statistically significant compared with group 1 ($P < .05$), † group 5 statistically significant compared with group 1 ($P < .05$). B, The NEFA levels during the first 20 minutes of the OGTT. M indicates the rate of disappearance of NEFAs from plasma (in micromoles per liter per minute).

(<100 mg/dL). There was no statistical variance in the systolic BP levels among all the subjects. Most of the subjects with NGT (62.5%), IGT (66.7%), and IPH (72.7%) had elevated levels of systolic BP (≥130 mm Hg), with a clear trend of progressively increasing differences from the NGT to IPH. Simultaneously, only 18% of subjects with NGT and 20% in both groups with IGT and IPH had elevated diastolic BP ≥85 mm Hg. The levels of HbA_{1C} and urinary microalbumin had a tendency to increase through groups NGT, IGT, and IPH; but the values were not statistically different. The total cholesterol levels, high-density lipoprotein, low-density lipoprotein, triglycerides, percentage of total body and truncal fat mass, and percentage of total lean body mass were similar in these groups.

3.2. Plasma fasting glucose and hormone measurements

The plasma fasting glucose levels in subjects with NGT, IGT, and IPH were 84.6 ± 1.8, 90 ± 1.8, and 91.8 ± 1.8 mg/dL, respectively (Fig. 1A). The plasma fasting insulin levels of 42 ± 7 pmol/L for NFG/NGT, 32 ± 5 pmol/L for IGT, and 39 ± 5 pmol/L for IPH did not differ statistically (Fig. 1B). Fasting incretin levels (GLP-1 and GIP) were similar in all 3 groups (Table 2).

We measured fasting plasma levels of C-peptide, leptin, ghrelin, adiponectin, and resistin for all subjects (Table 2); and no significant differences were found. However, leptin and adiponectin levels seem to be elevated in the NGT subjects compared with the subjects with IGT and IPH. Interleukin 12 was the only cytokine with significantly higher levels in the IPH group compared with the other 2 groups. The TNF-α, TNF-αsRI, TNF-αsRII, and IL-6 plasma cytokine levels were also measured in the fasting state; and although TNF-α and TNF-αsRI demonstrated a tendency to be higher in subjects with IPH, the elevation was not statistically significant. Similarly, C-reactive protein levels did not differ statistically between the 3 groups but once again had a tendency to be higher in subjects with IPH (Table 3).

The plasma fasting NEFA concentrations (550 ± 60, 700 ± 60, and 800 ± 70 μmol/L for subjects with NGT, IGT, and IPH, respectively) were statistically higher in IPH compared with NGT (P = .01); and levels in IGT were between the other 2 groups (Fig. 2A).

3.3. Response to OGTT

By 15 minutes after OGTT, IGT and IPH subjects already had significantly higher plasma glucose levels compared with NGT (Fig. 1A). Subjects with IGT and IPH also tended to be increasingly hyperinsulinemic when compared with NGT for the 2 hours of the OGTT (Fig. 1B); however, the areas under the curves for insulin were not statistically different between the groups (25 944 ± 2751, 31 666 ± 2751, and 33 969 ± 4941 pmol/[L·min] for NGT, IGT, and IPH, respectively). At the 2-hour time point, plasma insulin levels were significantly higher in the IPH group compared with NGT. Because of similar FPG and insulin levels, HOMA-IR was not statistically different between the 3 groups. Interestingly, the $II_{0\text{-}20\ minutes}$ had a tendency to decline through the 3 groups; but the decrease was not statistically significant. Both the MCR and the ISI were able to detect declining insulin sensitivity across and between all 3 groups (P < .05). The OGIS for NGT subjects was significantly better compared with those in the other 2 groups (P < .05); the other 2 groups, however, using OGIS, were similar to each other (Table 4). In addition, the calculated β-cell function during first- and second-phase insulin secretion was significantly better in NGT subjects compared with those in the IGT and IPH subjects (P < .05, Table 4).

There was no significant difference in plasma GIP and GLP-1 levels among all the subjects during the OGTTs (data not shown).

The plasma NEFA concentrations were also measured at 5, 10, 15, 20, 40, and 120 minutes. Plasma NEFA concentrations differed between IPH and NGT for the duration of the OGTT, with values for IGT consistently falling between the other 2 (Fig. 2A). The rate of the decrease in NEFAs from the plasma demonstrated statistical differences between all 3 groups during the first 20 minutes of the OGTT, in keeping with the decreased calculated β-cell function during first-phase insulin secretion (Fig. 2B, Table 4). The NEFA clearance rate was 3.0 μmol/(L·min) in IPH subjects, 7.6 μmol/(L·min) in IGT subjects, and

Table 4
Estimated insulin secretion and insulin sensitivity indices

	Group 1 (NGT) (n = 15)	Group 2 (IGT) (n = 12)	Group 5 (IPH) (n = 11)
HOMA-IR (μIU/mL·mmol/L)	0.5 ± 0.1	0.4 ± 0.1	0.5 ± 0.1
$II_{0\text{-}20\ min}$	0.75	0.64	0.45
ISI (μmol/[kg^{-1}·min^{-1}]·pmol/L)	0.10 ± 0.0	0.08 ± 0.00 *	0.06 ± 0.00 **, ***
MCR (mL/[kg^{-1}·min^{-1}])	8.9 ± 0.2	7.5 ± 0.3*	5.8 ± 0.3**,***
β-Cell function–1st phase (pmol/L)	1101.1 ± 59.2	572.3 ± 70.7*	363.6 ± 85.1**
β-Cell function–2nd phase (pmol/L)	266.6 ± 17.8	134.4 ± 18.0*	67.7 ± 24.5**,***
OGIS (mL/[min^{-1}·m^{-2}])	456.7 ± 11.8	384.9 ± 21.7 *	389.1 ± 16.5**

All values are expressed in means ± SEM.

* Group 2 statistically significant compared with group 1 (P < .05).

** Group 5 statistically significant compared with group 1 (P < .05).

*** Group 5 statistically significant compared with group 2 (P < .05).

11.9 μmol/(L·min) in NGT subjects. Furthermore, plasma insulin concentration that resulted in a 50% reduction in plasma NEFA levels was significantly higher in subjects with IPH (249 pmol/L at 40 minutes) and IGT (249 pmol/L at 40 minutes) compared with those with NGT (211 pmol/L at 20 minutes) ($P = .01$).

4. Discussion

Our study provides new insights into the pathophysiology of postprandial hyperglycemia in the elderly. After OGTT, plasma insulin levels in the 3 groups of subjects (NGT, IGT, and IPH) were similar during the first 20 minutes; but plasma glucose rose significantly higher in the IPH and IGT groups during that time. Clearly, this suggests that the insulin sensitivity during the first 20 minutes after OGTT was inadequate in the IGT and IPH groups and that it was at least one determinant of the subsequent elevated 2-hour glucose values of these 2 groups. The abnormal glucose levels during the OGTT could not be effectively offset, despite increasing plasma insulin concentrations during the "second phase" in subjects with impaired or diabetic 2-hour plasma levels. Reliance only on the fasting glucose and insulin values therefore does not give a complete picture of glucose homeostasis in the elderly; fasting glucose and insulin levels, and consequently HOMA-IR, could not uncover any defect in glucose regulation in IPH. We therefore attempted to uncover what might be the cause(s) of the defective glucose homeostasis in the elderly BLSA subjects that was only evident when the OGTT was carried out. Of all the known factors associated with insulin resistance for which we tested, only the elevated NEFA fasting levels, in addition to the defective decay curve of plasma NEFAs after OGTT, seemed likely to be pathological factors.

Lipolysis, a process by which NEFAs are released from stored triglycerides, is known to be exquisitely sensitive to suppression by insulin [21,22]. Although fasting levels of insulin were similar in all 3 groups, fasting NEFAs were elevated in IGT and even more so in IPH. This implies that insulin suppression of lipolysis was less efficient in IGT and even less again in IPH. Complementing those findings in the fasting state is the finding that early insulin secretion after OGTT gave defective suppression of lipolysis in IGT and IPH, as determined by the slope of the rate of clearance of NEFAs in plasma, compared with NGT and the insulin levels needed to suppress lipolysis by 50% was greater in IGT and greater again in IPH. These findings favor the conclusion that lipolysis determines insulin sensitivity to glucose disposal in our subjects.

The NEFAs are known to influence glucose transport; and a causative link between elevated plasma NEFA concentrations, subsequent defective glucose transport, and development of type 2 diabetes mellitus has been shown [23]. They decrease insulin-mediated glucose transport by decreasing the activity of phosphatidylinositol 3–kinase, a key insulin-regulated enzyme essential in glucose transporter 4 translocation to the plasma membrane [24]. As the appearance of NEFAs into the plasma was not suppressed in the IPH subjects in the first 20 minutes (nor in the impaired subjects to a lesser extent) after the OGTTs, then the insulin-mediated glucose transporter 4 translocation in muscle and the rate of subsequent glucose disposal were likely compromised. In addition, NEFAs have been shown to induce hepatic insulin resistance by interfering with the ability of insulin to stimulate glucokinase activity [25]; and the unsuppressed NEFAs during the early part of the OGTTs would result in alteration in rates of gluconeogenesis and glucogenolysis [26]. Finally, it has been shown that reducing NEFAs overnight with acipimox, an antilipolytic drug, resulted in a doubling of insulin-mediated glucose uptake and reduced insulin resistance in obese subjects [27]. Therefore, we conclude that the elevated NEFAs are playing a key role in the pathogenesis of the elevated 2-hour plasma glucose levels after OGTT in IPH and to a lesser extent in IGT subjects.

Subjects with impaired and diabetic 2-hour plasma levels of glucose after OGTT exhibited decreased insulin sensitivity after challenge. Unlike HOMA-IR, the ISI and MCR (indices based on not only fasting plasma samples but also 2-hour values for glucose from an OGTT) clearly distinguished between fasting euglycemic subjects with normal and those with impaired or diabetic 2-hour glucose responses. In addition, as we found previously [17], they distinguished between impaired and diabetic 2-hour glucose responses. It has been shown that 0- and 2-hour OGTT plasma values for glucose and insulin are more reproducible than other time points; therefore, taking advantage of the end point values reveals differences even between subjects with similar intermediate plasma levels, given that their 2-hour values are different. The β-cell function indices also show that although insulin secretion clearly increased after OGTT in subjects with IGT and IPH compared with NFG/NGT, it was not sufficient to overcome the impairment in insulin sensitivity. This also implies an effect of the NEFAs on decreased β-cell responses. The elevated fasting levels of NEFAs lead us to assume that there is accumulation of triglycerides in β-cells during fasting, and this has been postulated to lead to reductions in adenosine triphosphate production from glucose [28,29].

Several studies, including many recent ones, have implicated a number of adipocyte-derived factors such as leptin, resistin, and adiponectin in mediating insulin resistance in patients with type 2 diabetes mellitus [30-32]. Tissue expression and concentrations of adiponectin in plasma have been shown to directly correlate with insulin sensitivity [33]. Adiponectin concentrations have been reported as being decreased in insulin-resistant states and type 2 diabetes mellitus [34], and they have been introduced as a predictor for subsequent development of type 2 diabetes mellitus in some populations. No statistically significant differences in plasma levels of the hormone were noticeable

between our subject groups. In humans, resistin is produced by macrophages localized in the adipose tissue in an inflammatory response [35]. Previous studies have shown elevated resistin levels in diabetes and have implicated resistin in the pathogenesis of obesity-mediated insulin resistance [31,36,37]. Fasting plasma resistin levels were also not statistically different between NGT, IGT, and IPH subjects. Furthermore, apart from plasma levels of IL-12, we did not see any difference in any of the cytokines and cytokine receptors that we measured; specifically, levels of TNF-α and IL-6, which have been repeatedly implicated in causing insulin resistance [38,39], were similar in all 3 groups. Interleukin 12 has been implicated in participating in autoimmune diabetes and β-cell destruction [40]; and therefore perhaps, the elevated plasma levels of IPH subjects are contributing to the ineffective β-cell responses. These data suggest that adipocyte-derived factors are not playing a role in the insulin resistance to lipolysis and glucose disposal seen in IGT and IPH subjects. However, the small size of our sample may not afford adequate power to detect differences between our groups.

Although fat mass and lean body mass were similar in all 3 groups, we could not address muscle quality in this study. Muscle composition may be different between the 3 groups, and an inverse relationship exists between intramuscular triglyceride level and insulin sensitivity in muscle that is thought to play a role in insulin-resistant states of obesity and type 2 diabetes mellitus [41]. Elevated fasting levels of NEFAs would lead to increased delivery to skeletal muscle, which would further decrease insulin sensitivity. The antilipolytic effect of insulin is thought to decrease with aging [42], but that cannot account for the decline seen with glucose intolerance in BLSA subjects because we age-matched the NGT subjects to the glucose-intolerant groups.

GLP-1 and GIP, collectively known as *incretins*, belong to the glucagon-secretin family of gastrointestinal peptide hormones. These hormones are synthesized in and released from specialized enteroendocrine L and K cells, respectively, in the small and large intestine in response to food intake; and they constitute vital mediators of food-stimulated, glucose-dependent insulin secretion [43,44]. By accounting for up to 60% of the insulin secretory response after an oral glucose load, they play important roles in promoting nutrient assimilation, regulating energy absorption, and maintaining glucose homeostasis [45]. Fasting levels of both incretins and secreted levels after OGTT were similar in all 3 groups; and therefore, it is not evident that they play any role in the abnormal 2-hour glucose levels of the impaired or diabetic groups.

When we followed IPH subjects into their next OGTT visit, we found encouraging clinical results when they used lifestyle changes. On return visits 1 or more years after being classified as IPH, 5 subjects had impaired or normal 2-hour glucose levels; all 5 had reduced BP, fasting resistin levels, BMI, and total body weight. The weight loss was a consequence of active reduction in their total calorie intake after being informed on a previous visit of the diabetic result. Therefore, it is never too late to change one's eating habits. One subject, who gained 22 lb in weight, had progressed to DFG/DGT (group 7). According to the data from this longitudinal study, IPH in the presence of NFG does not inevitably lead to DFG. We will continue to follow these groups.

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