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Contribution of Non-Esterified Fatty Acids to Insulin Resistance in the Elderly with Normal Fasting but Diabetic 2h Post Challenge Plasma Glucose Levels: Baltimore Longitudinal Study of Aging

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Abstract

Isolated postchallenge hyperglycemia (IPH) with normal fasting plasma glucose (NFG) <100mg/dl and plasma glucose with diabetic 2h plasma glucose (DGT) ≥ 200mg/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 them 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 (75g) on two or more occasions. We measured plasma levels of glucose, insulin, C-peptide, GLP-1, GIP, ghrelin, leptin, adiponectin, resistin, C-reactive protein, cytokines and their soluble receptors, as well as NEFAs. We determined that 22 subjects in BLSA had IPH, accounting for 2.1% of the BLSA population. All 22 were >65 yrs old. They were then matched by age, sex and BMI to 12 subjects who had isolated impaired glucose tolerance (IGT) and 15 subjects with normal glucose tolerance (NGT). All subjects had normal fasting glucose (NFG) levels <100mg/dl in accordance with American Diabetes Association (ADA): 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 two groups, except at the 2h time, when their insulin levels were higher than NGT (P<0.05). Although there was a clear trend for differences in the insulinogenic index (II_{0-20 min}), the areas under the curves for insulin, systolic BP, 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 three groups. NEFA levels were significantly elevated in the fasting state (P<0.05) in the IPH compared to NGT, with IGT intermediate between the other two groups. The rate of clearance of NEFAs after the OGTT decreased progressively from the NGT to the IPH group (NGT-11.9 vs. IGT-7.6 vs. IPH-3.0, $\mu\text{mol/l}\cdot\text{min}^{-1}$). We conclude that the rate of suppression of lipolysis in the elderly determines the sensitivity of glucose uptake to insulin after OGTT.

KEY TERMS

Glucose; Insulin; GLP-1; GIP; BMI; Insulin; Ghrelin; Leptin; Adiponectin; Resistin; Cytokines; NEFAs; Oral glucose tolerance test (OGTT); Baltimore Longitudinal Study of Aging (BLSA)

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Introduction

Diabetes is characterized biochemically by a fasting plasma glucose (FPG) level $\geq 126\text{mg/dl}$, a plasma glucose $\geq 200\text{mg/dl}$ 2h after OGTT, or both [1]. The number of subjects with IPH, defined by FPG $<126\text{mg/dl}$ and 2h plasma glucose $\geq 200\text{mg/dl}$, was estimated at 9.8 % in a national samples of US adults with type 2 diabetes (age 40–74 years) [2]. The prevalence of IPH in the Tehran Lipid and Glucose Study of non-diabetic subjects aged >20 years was 2.5% using a FPG of $<126\text{mg/dl}$, but using FPG of $<100\text{mg/dl}$, prevalence was only 0.54% [3]. The DECODE study (Diabetes Epidemiology: Collaborative Analysis of Diagnostic Criteria in Europe) concluded that one-third of the older subjects (60–79 years) with FPG $<126\text{mg/dl}$ also had IPH and, most important, subjects with IPH had an elevated risk of mortality that was similar to that of subjects with FPG $\geq 126\text{mg/dl}$ [4]. Several other studies concur with DECODE in estimating that IPH places people at risk for cardiovascular disease and mortality [5–7]. Moreover, previous results from BLSA determined that impaired glucose tolerance, but not impaired fasting glucose, 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. In order to remove any influence of impaired fasting glucose we restricted our IPH definition to only subjects with NFG/DGT, defining NFG as plasma glucose levels of $<100\text{mg/dl}$, based on ADA classification, 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 to 92 years old) with NFG in BLSA, we found 22 subjects with IPH, all above the age of 65 years. On the other hand, we were unable to find a single subject under the age of 65 years in the IPH group. From our data, it therefore appears that IPH with FPG $<100\text{mg/dl}$ 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 BMI of $26.4 \pm 1.0\text{kg/m}^2$ and an average age of 81.1 ± 2.2 years, this group has morphological traits more similar to non-diabetic subjects than to subjects with diabetic fasting glucose levels and diabetic glucose tolerance DFG/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 NGT. NFG depends on adequate basal insulin secretion and insulin sensitivity, mainly in the liver, in order to control hepatic glucose output. Abnormalities of these two requirements have been documented to be characteristic of impaired and diabetic fasting glucose levels [9]. On the other hand, impaired and diabetic 2h plasma glucose levels after an OGTT are thought to reflect peripheral insulin resistance [10]. We found that the IPH subjects had significantly elevated non-esterified 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.

Methods

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, D.C. 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 all 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 (0min), after which subjects drank 75g glucose in 300ml solution (SunDex; Fisherbrand, Pittsburgh, PA), and

blood samples were drawn at 5, 10, 15, 20, 40, 60, 80, 100, and 120min (2h) after oral administration [12,13]. Using the results of plasma glucose concentrations at 0min and 2h, we classified every BLSA subject into one of nine different groups, as shown in Table 1. Subjects with normal fasting glucose (NFG, $\leq 99\text{mg/dl}$) and normal 2h glucose tolerance (NGT, $\leq 139\text{mg/dl}$) belong to group 1. Subjects with NFG and impaired 2h glucose tolerance (IGT, $140\text{--}199\text{mg/dl}$), impaired fasting glucose (IFG, $100\text{--}125\text{mg/dl}$) and IGT, and IFG/NGT belong to groups 2, 3, and 4, respectively. Subjects with NFG and diabetic 2h glucose tolerance (DGT, $\geq 200\text{mg/dl}$), IFG/DGT, diabetic fasting glucose (DFG, $\geq 126\text{mg/dl}$)/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 $\geq$ two occasions: their age range was 66–91 years and BMI range was $20\text{--}30\text{kg/m}^2$. Thirty-six subjects had IGT on at least two occasions. When subjects had multiple visits and where the results of OGTTs were similar, results from last visit were used. We excluded subjects with hemolyzed plasma samples, missing plasma samples at three or more time points, and those who had taken steroids (i.e. prednisone) within three 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).

Plasma Hormone and Biochemical Assays

We quantified plasma glucose concentration levels using a glucose analyzer (Beckman Instruments, Brea, CA). We measured 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, NIA/NIH) using an Auto Analyzer (Synchron CX-5; Beckman Instruments). Anthropometric measurements, including weight, height, and blood pressure were recorded at the time of the visit, as previously described in BLSA [14]. Percent total body and truncal fat mass and percent total lean body mass was estimated. We measured 24-hour urinary microalbumin levels with an Array 360 System (Beckman). We assayed plasma samples for insulin, C-peptide and resistin by enzyme-linked immunosorbent assay (ELISA) (Alpco Diagnostics, Salem, NH) with intra-assay variations of 4.8–9.0%, 2.9–4.8%, and 2.8–3.4% and inter-assay variations of 2.6–3.6%, 0.6–4.8%, and 5.1–6.9%, respectively. We also assayed plasma samples for leptin, GLP-1, and GIP using ELISA (LINCO Research, St. Charles, MO) with intra-assay variations of 1.09–4.98%, 6.0–9.0%, and 3.0–8.8%, in addition to inter-assay variations of 3.89–5.33%, 7.0–13.0%, and 1.8–6.1%, respectively. We detected plasma IL-6sR, TNF- α sRI, and TNF- α sRII with ELISA (R & D systems, Minneapolis, MN) possessing intra-assay variations of 2.3–8.6%, 3.6–5.0%, and 3.6–5.0% and inter-assay variations of 6.5–9.6%, 3.7–8.8%, and 3.7–8.8%, respectively. We measured fasting plasma C-reactive protein levels by ELISA (Alpha Diagnostic International, San Antonio, TX) with an intra-assay variation of 2.1–4.5% and an inter-assay variation of 3.0–7.0%. The human cytokine chemokine panel (LINCO) 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, Hercules, CA) with an intra-assay precision of 8.6% and 9.0% and an inter-assay 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 (RIA) (LINCO) having intra-assay and inter-assay variation of 1.78–6.21% and 6.9–9.25%, respectively. In addition, we assayed plasma samples for total ghrelin using RIA (Phoenix Pharmaceuticals, Belmont, CA) with calculated intra-assay and inter-assay variations of 6.7% and 7.8%, respectively. Finally, we measured non-esterified free fatty acids in plasma using an enzymatic endpoint assay (WAKO Chemicals, Richmond VA) with a precision variable range from 1.1–2.7%.

Statistical Analysis

All values are shown as means $\pm$ sem. We compared the sex and race ratios of all groups with the chi-square test. We used a one-way ANOVA test and SAS (version 9.1, SAS Institute, Cary, NC) to compare body composition, hormone, blood pressure, and NEFA levels and modeled for insulin sensitivity and insulin secretion, while adjusting for age and BMI among all the groups. We quantified insulin sensitivity by calculating the homeo static model assessment of insulin resistance (HOMA_{IR}) using fasting plasma glucose and insulin levels [15] and assessed early phase insulin secretion by measuring the insulinogenic index ($\Pi_{0-20 \text{ min}}$) as the ratio of the increment in the plasma insulin level to that of the plasma glucose level during the first 20 min after a modified OGTT [16]. We also calculated the insulin sensitivity index (ISI), metabolic clearance rates (MCR), 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 min value] and 120) [20].

Results

Characteristics of Subjects

Characteristics of all the subjects are presented in Table 2. These subjects had similar age (66–92 yrs) and BMI (22–30kg/m²) ranges and fasting glucose levels (<100mg/dl). There was no statistical variance in the systolic blood pressure (BP) levels among all the subjects. The majority of subjects with NGT (62.5%), IGT (66.7%), and IPH (72.7%) had elevated levels of systolic BP (≥ 130 mmHg) with clear trend of progressively increase differences from the NGT to the 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, HDL, LDL, triglycerides, percent total body and truncal fat mass and percent total lean body mass were similar in these groups.

Plasma Fasting Glucose and Hormone Measurements

The plasma fasting glucose levels in subjects with NGT, IGT, and IPH were 84.6 $\pm$ 1.8mg/dl, 90 $\pm$ 1.8mg/dl, and 91.8 $\pm$ 1.8mg/dl respectively (Fig. 1a). The plasma fasting insulin levels of 42 $\pm$ 7pmol/l for NFG/NGT, 32 $\pm$ 5pmol/l for IGT, and 39 $\pm$ 5pmol/l for IPH did not differ statistically (Fig. 1b). Fasting incretin levels (GLP-1 and GIP) were similar in all three 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 to the subjects with IGT and IPH. IL-12 was the only one cytokine with significantly higher levels in IPH group compared to the other two groups. TNF- α , TNF- α RI, TNF- α RII and IL-6 plasma cytokine levels were also measured in the fasting state and while TNF- α and TNF- α RI 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 three groups but once again had a tendency to be higher in subjects with IPH (Table 3).

The plasma fasting NEFA concentrations (550 $\pm$ 60, 700 $\pm$ 60, and 800 $\pm$ 70 μ mol/l for subjects with NGT, IGT, and IPH respectively) were statistically higher in IPH compared to NGT (p=0.01), and levels in IGT were between the other two groups (Fig 2a).

Response to Oral Glucose Tolerance Test

By 15 min after OGTT, IGT and IPH subjects already had significantly higher plasma glucose levels compared to NGT (Fig 1a). Subjects with IGT and IPH also tended to be increasingly hyperinsulinemic when compared to NGT for the two hours of the OGTT (Fig 1b): however, the areas under the curves for insulin were not statistically different between the groups (25944 $\pm$ 2751, 31666 $\pm$ 2751, 33969 $\pm$ 4941 pmol/l·min⁻¹; NGT, IGT, IPH, respectively). At the 2h time point plasma insulin levels were significantly higher in the IPH group compared to NGT. Due to similar fasting plasma glucose and insulin levels, HOMA_{IR} was not statistically different between the three groups. Interestingly, the insulinogenic index (II_{0-20 min}) had a tendency to decline through the three groups, but the decrease was not statistically significant. Both the metabolic clearance rate (MCR) and the insulin sensitivity index (ISI) were able to detect declining insulin sensitivity across and between all three groups ($p < 0.05$). The oral glucose insulin sensitivity (OGIS) for NGT subjects was significantly better compared to the other two groups ($p < 0.05$); the other two 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 to IGT and IPH subjects ($p < 0.05$, 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 two (Fig. 2a). The rate of the decrease of NEFAs from the plasma demonstrated statistical differences between all three groups during the first 20 min of the OGTT, in keeping with the decreased calculated β -cell function during first phase insulin secretion (Fig. 2b, Table 4). NEFA clearance rate was 3.0 μ mol/l·min⁻¹ in IPH subjects, 7.6 μ mol/l·min⁻¹ in IGT subjects, and 11.9 μ mol/l·min⁻¹ 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 40min) and IGT (249 pmol/l at 40min), compared to those with NGT (211 pmol/l at 20min) ($p = 0.01$).

Discussion

Our study provides new insights into the pathophysiology of postprandial hyperglycemia in the elderly. Following OGTT, plasma insulin levels in the three groups of subjects (NGT, IGT, and IPH) were similar during the first 20 min, 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 20min post-OGTT was inadequate in the IGT and IPH groups and that it was at least one determinant of the subsequent elevated 2h glucose values of these two 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 2h 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, appeared likely 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]. Even though fasting levels of insulin were similar in all three 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 to NGT and the insulin levels needed to suppress lipolysis by 50% was greater in the IGT and greater again in IPH. These findings favor the conclusion that lipolysis determines insulin sensitivity to glucose disposal in our subjects.

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 has been shown [23]. They decrease insulin-mediated glucose transport by decreasing activity of phosphatidylinositol-3-kinase, a key insulin-regulated enzyme essential in GLUT4 translocation to the plasma membrane [24]. As the appearance of NEFAs into the plasma was not suppressed in the IPH subjects in the first 20min (nor in the impaired subjects to a lesser extent) after the OGTTs, then insulin-mediated GLUT4 translocation in muscle and the rate of subsequent glucose disposal was likely compromised. Additionally, 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 2h plasma glucose levels after OGTT in IPH and to a lesser extent in IGT subjects.

Subjects with impaired and diabetic 2h plasma levels of glucose after OGTT exhibited decreased insulin sensitivity post-challenge. Unlike $HOMA_{IR}$, the ISI and MCR (indices based on not only fasting plasma samples but also 2h values for glucose from an OGTT) clearly distinguished between fasting euglycemic subjects with normal and those with impaired or diabetic 2h glucose responses. Additionally, as we found previously [17], they distinguished between impaired and diabetic 2h glucose responses. It has been shown that 0 and 2h 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 2h values are different. The beta cell function indices also show that while insulin secretion clearly increased following OGTT in subjects with IGT and IPH compared to NFG/NGT, it was not sufficient to overcome the impairment in insulin sensitivity. This also implies an effect of the NEFAs on decreased beta cell responses. The elevated fasting levels of NEFAs leads us to assume that there is accumulation of triglycerides in beta cells during fasting, and this has been postulated to lead to reductions in ATP 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 [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 [34], and they have been introduced as a predictor for subsequent development of type 2 diabetes 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 three groups. IL-12 has been implicated in participating in autoimmune diabetes and beta cell destruction [40], and therefore perhaps the elevated plasma levels of IPH subjects are contributing to the ineffective beta 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, that small a size of our sample may not afford adequate power to detect differences between our groups.

While fat mass and lean body mass were similar in all three groups, we could not address muscle quality in this study. Muscle composition may be different between the three 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 [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 sixty percent of the insulin secretory response following 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 as well as secreted levels after OGTT were similar in all three groups, and therefore it is not evident that they play any role in the abnormal 2h 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 employed lifestyle changes. On return visits one or more years after being classified as IPH, five subjects had impaired or normal 2h glucose levels; all five had reduced blood pressure, fasting resistin levels, BMI, and total body weight. The weight loss was a consequence of active reduction of 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 pounds 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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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 (See Table 1) subjects. *Group 2 statistically significant compared to group 1 ($p < 0.05$), †Group 5 statistically significant compared to group 1 ($p < 0.05$), ‡Group 5 statistically significant compared to group 2 ($p < 0.05$).

FIG. 2.

Plasma NEFAs after administration of 75 g oral glucose (OGTT) to BLSA participants. a. NEFA levels in groups 1, 2 and 5 (See Table 1) subjects. *Group 2 statistically significant compared to group 1 ($p < 0.05$), †Group 5 statistically significant compared to group 1 ($p < 0.05$), b. NEFA levels during the first 20min of the OGTT. M=rate of disappearance of NEFAs from plasma ($\mu\text{mol/l}\cdot\text{min}^{-1}$).

Table 1**Classification of Glucose Tolerance**

Subjects with normal fasting glucose (NFG, =99mg/dl) and normal 2h glucose tolerance (NGT, =139mg/dl) belong to group 1. Subjects with NFG and impaired 2h glucose tolerance (IGT, 140–199mg/dl), impaired fasting glucose (IFG, 100–125mg/dl) and IGT, and IFG/NGT belong to groups 2, 3, and 4, respectively. Subjects with NFG and diabetic 2h glucose tolerance (DGT, =200mg/dl), IFG/DGT, diabetic fasting glucose (DFG, =126mg/dl)/DGT, DFG/IGT, and DFG/NGT are classified in groups 5, 6, 7, 8, and 9, respectively.

2 hour plasma post OGTT	=200mg/dl [11.1mmol/l]	NFG/DGT (group 5)	IFG/DGT (group 6)	DFG/DGT (group 7)
	140–199mg/dl [7.78–11.06mmol/l]	NFG/IGT (group 2)	IFG/IGT (group 3)	DFG/IGT (group 8)
	=139mg/dl [7.7mmol/l]	NFG/NGT (group 1)	IFG/NGT (group 4)	DFG/DGT (group 9)
		= 99mg/dl [5.5mmol/l]	100–125mg/dl [5.6–6.9mmol/l]	= 126mg/dl [7.0mmol/l]
Fasting plasma glucose levels				

Table 2

Characteristics of All the Subjects.

	Group 1 (NGT) (n=15)	Group 2 (IGT) (n=12)	Group 5 (IPH) (n=11)
Age (yrs)	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
24h 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)	131.0 +/- 5.1 (62.5)	137.8 +/- 3.8 (66.7)	142.7 +/- 4.7 (72.7)
Diastolic BP (% , BP = 80)	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

HbA_{1C} is expressed in means +/- SD. All other values are expressed in means +/- SEM.

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- α RI (pg/ml)	125.0 $\pm$ 10.2	117.7 $\pm$ 8.9	175.5 $\pm$ 32.3
TNF- α RII (pg/ml)	245.0 $\pm$ 16.8	247.5 $\pm$ 19.9	320.2 $\pm$ 41.8
IL-6 sR (pg/ml)	358.5 $\pm$ 24.7	343.7 $\pm$ 24.0	337.4 $\pm$ 36.1
IL-6 (pg/ml)	36.0 $\pm$ 17.4	13.4 $\pm$ 7.2	25.2 $\pm$ 8.0
IL-12 (pg/ml)	1.0 $\pm$ 0.3	2.8 $\pm$ 1.9	15.9 $\pm$ 6.8 ^{†‡}
TNF- α (pg/ml)	5.5 $\pm$ 0.8	5.1 $\pm$ 1.0	6.2 $\pm$ 1.2
C-reactive protein (ng/ml)	876.2 $\pm$ 158.4	1891.5 $\pm$ 709.5	2466.0 $\pm$ 1264.1

All values are expressed in means $\pm$ SEM.[†] Group 5 statistically significant compared to group 1 (p<0.05)[‡] Group 5 statistically significant compared to group 2 (p<0.05)

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
Insulinogenic Index _{O-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 ^{†‡}
Beta cell function - 1st phase (pmol/l)	1101.1 +/- 59.2	572.3 +/- 70.7 *	363.6 +/- 85.1 [†]
Beta 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 to group 1 (p<0.05)

[†] Group 5 statistically significant compared to group 1 (p<0.05)

[‡] Group 5 statistically significant compared to group 2 (p<0.05)