Insulin Kinetics, Insulin Action, and Muscle Morphology in Lean or Slightly Overweight Persons With Impaired Glucose Tolerance

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Glucose intolerance is influenced by body fat mass, as well as muscle fiber composition. To examine the relation between the metabolic profile and muscle morphology in this condition, we performed muscle biopsies and hyperglycemic clamps to determine insulin secretion and clearance, and the insulin effects on glucose disposal and nonesterified fatty acids (NEFA) in 45 glucose intolerant persons (body mass index [BMI], 27.8 ± 3.0 kg/m^2) and 45 normoglycemic controls (BMI, 25.8 ± 2.7 kg/m^2) (P = .001). After adjustment for BMI, glucose-intolerant subjects had lower first-phase insulin release (726 *v* 954 pmol/L, P = .04). Glucose-intolerant subjects and controls differed in fasting insulin, insulin clearance, and insulin sensitivity to glucose disposal before, but not after, standardizing for BMI. During the clamp, glucose-intolerant subjects had less NEFA suppression and elevated levels of NEFA compared with controls (85% ± 9% *v* 90% ± 6%, P = .02; and 70 ± 42 μmol/L *v* 45 ± 28 μmol/L, P = .01). Glucose-intolerant subjects also had a higher percentage of insulin-insensitive, type 2b muscle fibers, which are not adapted for fat oxidation (7% ± 9% *v* 9% ± 9%, P = .003). BMI was not associated with NEFA suppression or the percentage of type 2b muscle fibers in either group. In conclusion, glucose-intolerant persons have impaired first-phase insulin release, an elevated percentage of type 2b muscle fibers, and increased NEFA availability. Reduced insulin clearance, hyperinsulinemia, and insulin resistance were associated with small increments in BMI.

A WIDE RANGE of abnormalities in the regulation of glycemic control have been observed in persons with impaired glucose tolerance.[1-3] Some studies reported impairment in peripheral insulin sensitivity and compensatory hyperinsulinemia,[1,4-6] together with impaired pancreatic β-cell function and development of non–insulin-dependent diabetes (NIDDM).[7-9] Other studies found abnormalities in the biphasic insulin response to intravenous glucose,[10] such as defect first-phase insulin release from β cells[11,12] and hyperinsulinemia in second-phase insulin release.[12] Most studies demonstrate a link between obesity and impaired glucose tolerance.[3,9,13-15]

High proportions of body fat increase the turnover of nonesterified fatty acids (NEFA),[16,17] which impairs glucose utilization by the fatty acid/glucose cycle.[18] Skeletal muscle is an important site for glucose utilization, as up to 70% of an orally administered glucose load is metabolized in muscle tissue.[19] NEFA disposal occurs primarily in muscle and liver.[20] Muscle fiber composition may therefore influence both muscle glucose and fat utilization. White, fast-twitch, type 2b muscle fibers are characterized by low insulin sensitivity,[21] high glycolytic and low oxidative capacity, and reduced capillary supply, and are not adapted to fat oxidation.[22] In contrast, red, slow-twitch, type 1 muscle fibers are insulin-sensitive,[21] have high oxidative capacity, and are well supplied with capillaries.[22] The fast-twitch, type 2a muscle fibers contain both oxidative and glycolytic fibers. Increased proportions of type 2b fibers have been found in the muscles of obese persons[23] and in persons with NIDDM.[24] The percentage of type 2b muscle fibers has been associated with the amount of body fat,[25,26] waist-to-hip ratio,[23,26] levels of plasma glucose[25] and insulin,[24,26] and insulin sensitivity.[23,26]

The present study was performed to examine whether impaired glucose tolerance in lean or slightly overweight persons is linked with abnormalities in insulin delivery, insulin efficiency, and muscle morphology. The metabolic profile and muscle morphology in 45 glucose-intolerant persons and 45 normoglycemic controls were studied for comparison of insulin secretion, insulin clearance, insulin sensitivity for glucose disposal and suppression of NEFA, and muscle fiber distribution.

METHODS

Subjects

Ninety persons (57 males and 33 females) who participated in a population screening in 1987 (The Tromsø Health Survey[27]) were recruited to the present study. Forty-five subjects had fasting plasma glucose levels less than 7.8 mmol/L and 2-hour postload glucose levels between 7.8 and 11.1 mmol/L after an oral load of 1 g of dextrose per kilogram body weight, to a maximum of 75 g dextrose. Forty-five subjects with fasting glucose levels less than 6.0 mmol/L and postload glucose levels less than 7.8 mmol/L served as normoglycemic controls. Fifty-two subjects had mild, untreated hypertension (systolic blood pressure < 190 mm Hg, diastolic blood pressure > 90 mm Hg and < 110 mm Hg). None had type II diabetes or ischemic heart disease and none were on medication. None had a body mass index (BMI) greater than 30 kg/m^2 and all appeared healthy on clinical examination. Each participant completed a questionnaire about physical activity, smoking, and alcohol habits. The study was approved by the Regional Board of Research Ethics. All subjects gave written informed consent to participate in the study. Metabolic studies were started at 8 AM after an overnight fast. Muscle biopsies were performed within 1 week after the metabolic studies.

Clinical and Laboratory Measurements

Body weight was measured in light clothing without shoes. The same weight was used for all participants. The waist-to-hip ratio was calculated as body circumference at the level midway between the inferior border of the rib cage and superior border of the iliac crest, divided by the maximal circumference of the buttocks.[28] Measurements were determined early in the morning after an overnight fast.

Blood was arterialized by placing the hand in a heating device,[29] and

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blood samples were drawn from a cannulated dorsal hand vein. Plasma glucose was analyzed bedside with a Yellow Spring Instruments glucose analyzer (2300 STAT PLUS; Yellow Springs, OH). All other blood samples were stored at −70°C. Plasma insulin,[30] C-peptide,[31] and proinsulin[32] were measured as previously described.[33,34] Serum NEFA were analyzed by an acyl-coenzyme A oxydase–based colorimetric kit (Wako Nefa C Kit, Osaka, Japan). Serum triglycerides were measured on a Hitachi 737 Automatic Analyzer, Tokyo, Japan, with a kit from Boehringer Mannheim, Mannheim, Germany.

Insulin kinetics and action were assessed with an oral glucose tolerance test and a hyperglycemic clamp. Plasma glucose, insulin, and C-peptide responses after the oral glucose challenge were calculated as the arbitrary incremental area units over the 2-hour sampling time. As an approximate estimate of insulin clearance during the oral glucose tolerance test, we assessed the molar ratio of the area under the C-peptide curve: the area under the insulin curve.[35,36]

Hyperglycemic clamps[37,38] were used to measure the early insulin peak released from pancreatic β cells (first-phase insulin release), the steady-state insulin plateau during sustained glucose stimulation (second-phase insulin release), the approximate insulin clearance during intravenous glucose stimulation, insulin sensitivity to glucose disposal, and suppression of plasma NEFA under physiologic hyperinsulinemia, mimicking the postprandial situation. Plasma glucose levels were kept at 10 mmol/L for 3 hours by a variable glucose infusion, and blood samples for insulin and C-peptide measurements were drawn at −30, 0, 2.5, 5, 7.5, 10, 15, 20, 40, 60, 80, 100, 120, 140, 160, and 180 minutes. First-phase insulin release was calculated as the area under the insulin curve over the initial 10-minute sampling time. The approximate insulin clearance during this 10-minute period was calculated as the molar ratio of the area under the C-peptide curve: the area under the insulin curve. Second-phase insulin release was calculated as the area under the insulin curve from 120 to 180 minutes of the clamp period, and an estimate of insulin clearance was similarly calculated for this period. Insulin sensitivity to glucose disposal was expressed as the insulin sensitivity index, calculated by dividing the mean glucose infusion rate at 120, 140, 160, and 180 minutes of the clamp (μmol/kg/min) by the average plasma insulin concentration (pmol/L) during the same period. Suppression of plasma NEFA during physiologic glucose and insulin stimulation was expressed as the percent suppression of NEFA during the third hour of the hyperglycemic clamp, calculated by the formula: $((\text{[NEFA]}_{-30\text{-}0} - \text{[NEFA]}_{120\text{-}180})/\text{[NEFA]}_{-30\text{-}0}) \cdot 100$, where $\text{[NEFA]}_{-30\text{-}0}$ is the mean baseline concentration at 30 and 0 minutes before initiation of the clamp, and $\text{[NEFA]}_{120\text{-}180}$ is the mean NEFA concentration at 120 and 180 minutes of the clamp. We also performed a euglycemic, hyperinsulinemic clamp[37] in 43 participants to confirm that the insulin sensitivity indexes obtained from the two clamp techniques were comparable.[38] The insulin sensitivity indexes calculated by the two clamp techniques were highly correlated ($r = .77$, $P = .0001$); the slope (±SE) of the regression line was 2.44 ± 0.37 ($P = .0001$), and the intercept was −0.02 ± 0.04 ($P = 0.5$).

Muscle Biopsy

Muscle biopsies were obtained from the right vastus lateralis muscle with a percutaneous needle biopsy (modified Bergström needle[39]) under local anesthesia. The samples were trimmed, mounted, frozen in isopentane-cooled liquid nitrogen, and stored at −70°C. Transverse 10-μm thick sections were cut with a cryotome at −20°C. For analysis of muscle fiber composition, sections were stained for their myofibrillar adenosine triphosphatase activity[40] after preincubation at pH 4.3 and 9.4. The three fiber types were classified according to their staining characteristics in nonselected visual fields and in the same section.

Fiber area and fiber roundness were measured with fully automated morphometric techniques (Computer-aided microscopy, Quantimet 500+; Leica, Cambridge, UK). Fiber roundness, a measure of nonspecific myopathies,[41] was calculated as the ratio of fiber perimeter squared to fiber area. A decreased ratio reflects an increased degree of roundness. The mean of the values found for roundness of type 1, type 2a, and type 2b fibers is expressed as the mean fiber roundness of all fiber types. The average of the fiber areas measured for the three fiber types is expressed as the mean fiber area. The mean (SD) number of fibers counted and measured per biopsy was 352 ± 148. (magnification, ×6.3).

The percentage of perimysial connective tissue space, reflecting myopathy,[42] was determined as the mean of the percentages of connective tissue area compared with total muscle tissue area, measured in four artifact-free visual fields (magnification, ×10) with densitometric analysis of Gomori trichrome-stained sections[43] (Quantimet 500+; Leica).

Capillary density was estimated in hematoxylin-stained sections (magnification, ×16). Extracellular nuclei account for the nuclei in capillary endothelial cells, and may be used to estimate the degree of capillarization. The numbers of extracellular nuclei and muscle fibers were counted in artifact-free areas of 0.15 mm^2. The mean (±SD) number of fibers counted for measurement of capillarization was 22 ± 9. The average area of these fibers was measured with Quantimet 500+. Capillary density data were expressed as the number of extracellular nuclei per square millimeter muscle tissue, the number of extracellular nuclei per muscle fiber, and the muscle fiber area (μm^2) per extracellular nuclei.

All morphologic measurements were performed by one of the authors (I.T.), who was blinded to case status.

Statistical Analyses

All data were checked with regard to their frequency distribution, and transformed to normal distribution by logarithmic transformation when appropriate. Two-sample *t* tests were used to test differences between the glucose-intolerant group and the normoglycemic control group. Associations between BMI and the metabolic variables were examined in the two groups separately using Pearson's correlation coefficients and simple linear regression analysis. To test for an interaction between glucose tolerance (yes/no) and BMI, regression analysis was performed on the full dataset with the product term of glucose tolerance (yes/no, coded as 0 or 1) and BMI (kg/m^2), as assessed with a model that also included both terms separately. Analysis of covariance (ANCOVA) with BMI as a covariate was used to adjust for the difference in mean BMI among persons with impaired glucose tolerance and normal glucose tolerance. Independent relations were also examined in multiple linear regression models. Frequency differences in categorical variables obtained from the questionnaire were tested with the chi-square test. Results are given as the mean ±SD unless otherwise noted. A two-sided *P* value less than .05 was considered statistically significant. Data were analyzed using the SAS software package (Statistical Analysis System, Cary, NC).[44]

RESULTS

The glucose-intolerant participants differed from the normoglycemic control group in BMI ($P = .001$), systolic ($P = .01$) and diastolic ($P = .05$) blood pressure, fasting levels of plasma glucose ($P = .002$) and insulin ($P = .05$), and integrated responses of glucose ($P = .0001$) and insulin ($P = .05$) during the oral glucose tolerance test (Table 1). Waist-to-hip ratio did not differ significantly between the groups. No group differences were found for physical activity (Table 1), smoking habits, and alcohol intake (data not shown).

In both glucose-intolerant and normoglycemic persons, BMI was highly correlated with the measures of insulin kinetics and insulin action, and the slope of the correlation was similar in the two groups (Table 2). BMI and waist-to-hip ratio were intercor-

Table 1. Characteristics

Characteristic	Persons With Impaired Glucose Tolerance	Persons With Normal Glucose Tolerance
No.	45	45
Gender (M/F)	26/19	31/14
Age (yr)	57.4 ± 5.8	54.3 ± 9.5
BMI (kg/m²)	27.8 ± 3.0	25.8 ± 2.7†
Waist-to-hip ratio	0.99 ± 0.12	0.95 ± 0.11
Systolic blood pressure (mm Hg)	144 ± 20	134 ± 21*
Diastolic blood pressure (mm Hg)	90 ± 12	86 ± 12*
Fasting glucose level (mmol/L)	5.8 ± 0.8	5.4 ± 0.43†
Fasting insulin level (pmol/L)	64 ± 53	48 ± 16*
Area under the glucose curve (mmol/L)	16.9 ± 4.5	11.0 ± 3.2‡
Area under the insulin curve (pmol/L)	1,106 ± 682	813 ± 466*
Physical activity, leisure (%)		
Inactive (<0.5 h/wk)	49	42
Some (0.5-2 h/wk)	36	49
Regular (>2 h/wk)	15	9

NOTE. Data are means ± SD.
*$P < .05$.
†$P < .01$.
‡$P < .001$.

related in both groups (Table 2). NEFA levels and NEFA suppression during the clamp were not associated with BMI (data not shown). Blood pressure, gender, and age were not associated with any of the metabolic variables in the present study (data not shown).

Insulin Kinetics

Fasting plasma levels of insulin and proinsulin were higher in the glucose-intolerant group than in the normoglycemic group (Table 3), but the differences did not remain significant when BMI was included as a covariate. Insulin release during the initial 10 minutes of the hyperglycemic clamp was lower in the glucose-intolerant group compared with the normoglycemic control group; the difference was statistically significant when controlling for the group difference in BMI (Table 3). Insulin release during the third hour of the hyperglycemic clamp did not differ significantly between groups. The approximate insulin clearance, assessed as the peripheral C-peptide:insulin molar ratio after oral glucose stimulation, was lower in the glucose-intolerant group (2.3 ± 1.5) compared with normoglycemic controls (2.9 ± 1.5) ($P = .01$); the adjusted means did not differ significantly (Table 3). The C-peptide:insulin ratios during the initial 10 minutes of the hyperglycemic clamp and during the third hour of the clamp were 2.7 ± 1.3 and 3.6 ± 2.1 in the glucose-intolerant group, compared with 2.6 ± 2.1 ($P = .30$) and 4.1 ± 2.8 ($P = .10$) in the normoglycemic group. The differences were not significant when BMI was included as a covariate.

In the total study population ($N = 90$), the C-peptide:insulin ratio after oral glucose challenge correlated inversely with BMI ($r = -.52$, $P = .0001$), waist-to-hip ratio ($r = -.28$, $P = .01$), triglycerides ($r = -.28$, $P = .009$), and fasting NEFA levels ($r = -.22$, $P = .05$). Of these variables, only BMI remained significantly ($P = .0001$) associated with postload C-peptide: insulin ratio in a multiple linear regression analysis.

Insulin Action

The insulin sensitivity index for glucose disposal was lower in the glucose-intolerant group than the normoglycemic group, but the difference was not significant when BMI was included as a covariate (Table 3). The correlation between insulin sensitivity index and BMI in the total study group ($N = 90$) was $r = -.56$, $P = .0001$ (Fig 1).

The decrease in plasma NEFA concentrations during physiologic hyperglycemia and insulin stimulation, assessed as the percentage NEFA suppression during the hyperglycemic clamp, was decreased in the glucose-intolerant group (85% ± 9% *v* 90% ± 6%, $P = .02$; Table 3). The corresponding NEFA levels during insulin stimulation were 70 ± 42 μmol/L in the intolerant group and 45 ± 28 μmol/L in the control group ($P = .01$). Adjustment for differences in the insulin plateaus achieved during the hyperglycemic clamp did not influence the results. Fasting NEFA levels did not differ significantly (Table 3). Triglyceride levels were significantly higher in the glucose-intolerant group than in the control group, also when BMI was taken into account (Table 3).

Table 2. Correlations Between BMI and Variables Related to Insulin Kinetics and Insulin Action in Persons With Impaired (n = 45) and Normal (n = 45) Glucose Tolerance

	BMI				
	Persons With Impaired Glucose Tolerance		Persons With Normal Glucose Tolerance		Interaction
Variable	*r*	β (SE)	*r*	β (SE)	(*P* value§)
Waist-to-hip ratio	.50‡	.02 ± .01	.31*	.01 ± .01	.36
Fasting glucose	.36*	.07 ± .03	.46†	.07 ± .02	.96
Fasting insulin	.50‡	.08 ± .02	.44*	.05 ± .01	.21
Fasting proinsulin	.30*	.08 ± .03	.35*	.09 ± .04	.77
Fasting triglycerides	.28*	.04 ± .02	.34*	.02 ± .02	.73
1st-phase insulin	.25*	.04 ± .03	.27*	.06 ± .03	.82
2nd-phase insulin	.41†	.10 ± .03	.38†	.08 ± .03	.54
Insulin clearance¶	−.54‡	−.09 ± .02	−.35*	−.05 ± .02	.28
ISI	−.57‡	−.15 ± .03	−.44†	−.08 ± .02	.15

NOTE. *r* indicates Pearson correlation coefficient between BMI and waist-to-hip ratio or the metabolic variables. β (SE) indicates the corresponding regression coefficient (standard error) from simple linear regression with BMI as an independent variable. 1st-phase insulin: insulin release during the initial 10 minutes of a hyperglycemic clamp; 2nd-phase insulin, insulin release at 120 to 180 minutes during hyperglycemic clamp; ISI, insulin sensitivity index for glucose disposal during 120 to 180 minutes of a hyperglycemic clamp (μmol/kg/min/pmol/L). Data for insulin levels, triglyceride levels, insulin clearance, and insulin sensitivity index were logarithmically transformed.

*$P < .05$.
†$P < .01$.
‡$P < .001$.
§*P* value for product term of glucose tolerance (2 categories) and BMI in linear regression analysis.
¶The incremental area under the C-peptide curve (pmol/L) divided by the incremental area under the insulin curve (pmol/L) during the 2 hours sampling time of an oral glucose tolerance test, used as an approximate indication of hepatic insulin extraction.

Table 3. Fasting Insulin Levels, First- and Second-Phase Insulin Release, C-Peptide:Insulin Ratio, and Insulin Action in Persons With Impaired (n = 45) and Normal (n = 45) Glucose Tolerance

Variable	Persons With Impaired Glucose Tolerance		Persons With Normal Glucose Tolerance	
	Unadjusted Mean ± SD	Adjusted* Mean	Unadjusted Mean ± SD	Adjusted* Mean
Fasting insulin levels (pmol/L)	64 ± 53	60	48 ± 16†	52
Fasting proinsulin levels (pmol/L)	7.5 ± 6.9	7.0	4.7 ± 3.6‡	5.1
First-phase insulin release (pmol/L)	802 ± 606	726	910 ± 644	954†
Second-phase insulin release (pmol/L)	1,013 ± 744	940	857 ± 504	930
C-peptide:insulin ratio after oral glucose challenge	2.3 ± 1.5	2.5	2.9 ± 1.4†	2.6
Insulin sensitivity index	0.17 ± 0.17	0.18	0.22 ± 0.10‡	0.19
NEFA suppression during hyperglycemic clamp (%)	85 ± 9	85	90 ± 6†	90†
NEFA levels during hyperglycemic clamp (μmol/L)	70 ± 42	69	45 ± 28†	48†
Fasting NEFA levels (μmol/L)	497 ± 158	490	437 ± 154	451
Fasting triglyceride levels (mmol/L)	1.45 ± 0.79	1.41	1.09 ± 0.56‡	1.14†

NOTE. First- and second-phase insulin release: Areas under the insulin curve during the initial 10 minutes (first phase) and during 120 to 180 minutes (second phase) of the hyperglycemic clamp. C-peptide: insulin ratio after oral glucose challenge: the incremental area under the C-peptide curve (pmol/L) divided by the incremental area under the insulin curve (pmol/L) during the 2-hour sampling time of an oral glucose tolerance test. Insulin sensitivity index for glucose disposal is calculated as μmol glucose/kg body weight/min/pmol/L insulin during 120 to 180 minutes of the hyperglycemic clamp. NEFA suppression: percent reduction of NEFA levels at 120 to 180 minutes during hyperglycemic clamp, compared with baseline NEFA levels.

*Adjusted by ANCOVA for BMI.

†$P < .05$.

‡$P < .01$.

Muscle Morphology

Muscle fiber composition was not associated with BMI. The glucose-intolerant group had a higher percentage of type 2b fibers (9% ± 9%) compared with the control group (7% ± 9%) ($P = .003$), whereas the percentages of type 1 and 2a fibers, as well as the ratio of type 1 to type 2 fibers, did not differ in the groups (Table 4). In the total study group ($N = 90$), the percentage of type 2b muscle fibers correlated with NEFA suppression during the hyperglycemic clamp ($r = -.25$, $P = .05$), NEFA levels during the hyperglycemic clamp ($r = .30$, $P = .01$), and fasting plasma glucose ($r = .24$, $P = .03$). The percentage of type 2b fibers did not correlate with the insulin sensitivity index for glucose disposal.

Type 1 fiber area was greater in the glucose-intolerant group (5,135 ± 1,625 *v* 4,466 ± 1,536 μm^2; $P = .04$). Type 2a and 2b fiber area and mean fiber area of all fiber types did not differ in the groups (Table 4). No differences in muscle fiber roundness or in the percentage of perimysial connective tissue were observed (Table 4).

The estimated number of capillaries per square millimeter of muscle tissue was lower in the glucose-intolerant group (201 ± 49) compared with the control group (238 ± 91) ($P = .04$), whereas the estimated number of capillaries per muscle fiber and fiber area per capillary were similar (Table 5). No associations were found between capillarization data and the metabolic variables.

DISCUSSION

In the present study, subjects with impaired glucose tolerance had a higher BMI than the control group, and the results of the present study suggest that small differences in BMI index have a physiologic impact on insulin and glucose homeostasis.

We found that raising the peripheral blood sugar concentration to a level of 10 mmol/L by intravenous glucose infusion seemed to give a lower early insulin peak in the glucose-intolerant group compared with controls, but the difference was not significant. When controlling for BMI, first-phase insulin release was significantly lower in the glucose-intolerant group. The finding of decreased early β-cell insulin release in subjects

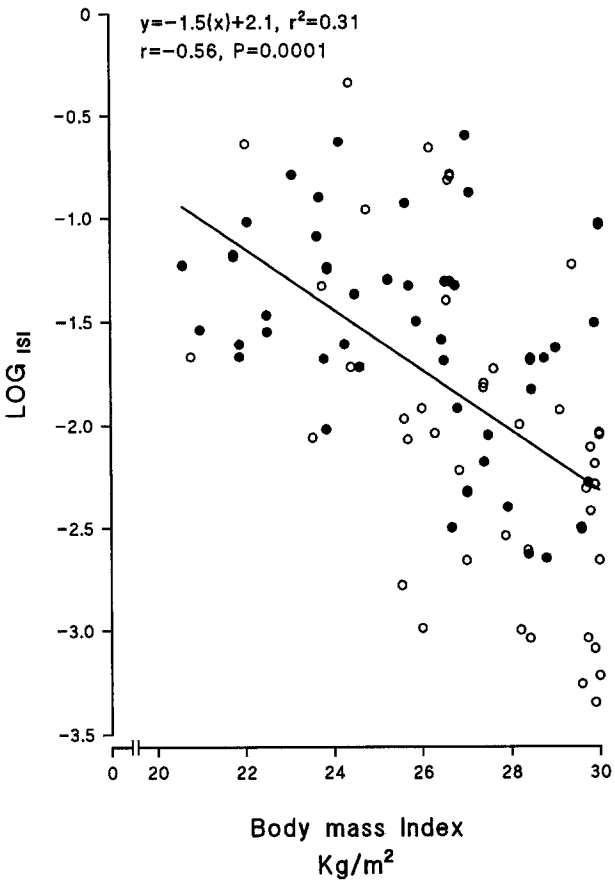

Fig 1. Relationship between insulin sensitivity (ISI) for glucose disposal and BMI. Values for ISI are transformed to normal distribution by logarithmic transformation. (●) Normal glucose tolerance (n = 45); (○) impaired glucose tolerance (n = 45).

Table 4. Muscle Fiber Composition and Morphology in Persons With Impaired (n = 45) and Normal (n = 45) Glucose Tolerance

Fiber Type	Persons With Impaired Glucose Tolerance	Persons With Normal Glucose Tolerance
Type 1		
%	54 ± 14	58 ± 12
Area (μm²)	5,135 ± 1,625	4,466 ± 1,536*
Roundness	1.56 ± 0.16	1.53 ± 0.11
Type 2a		
%	37 ± 12	36 ± 10
Area (μm²)	5,509 ± 2,020	5,254 ± 1,988
Roundness	1.53 ± 0.11	1.52 ± 0.09
Type 2b		
%	9 ± 9	7 ± 9†
Area (μm²)	4,604 ± 1,850	4,212 ± 1,517
Roundness	1.55 ± 0.16	1.54 ± 0.12
Ratio of type 1 to type 2 fibers	1.48 ± 1.28	1.52 ± 0.82
Fiber area (μm²), all fiber types	5,093 ± 1,628	4,721 ± 1,644
Fiber roundness, all fiber types	1.54 ± 0.12	1.53 ± 0.08
Perimysial connective tissue (%)	8 ± 4	9 ± 5

NOTE. Fiber roundness: ratio of fiber perimeter squared to fiber area. All muscle fiber data refer to musculus vastus lateralis.

*$P < .05$.

†$P < .01$.

with impaired glucose tolerance confirms the results of several previous reports.[2,3,11,12,45,46] The decreased early insulin response was not reflected by the area under the insulin curve after oral glucose challenge, which was found to be elevated in the glucose-intolerant group, probably due to the corresponding decrease in estimated insulin clearance. BMI was highly associated with the postload C-peptide:insulin ratio, and it is possible that glucose-intolerant subjects had reduced insulin clearance and increased postload peripheral insulin concentrations because they were slightly overweight compared with the control group. Enlarged body fat mass increases the amount of circulating NEFA through NEFA mobilization from, eg, 20 to 30 kg extra fat tissue.[16] The flux of NEFA to the liver will thereby increase. Central obesity is of particular importance for the hepatic NEFA supply, since abdominal fat tissues have high sensitivity for the lipolytic action of catecholamines.[47] Some

Table 5. Muscle Capillarization and Vessel Wall Thickness in Persons With Impaired (n = 45) and Normal (n = 45) Glucose Tolerance

Variable	Persons With Impaired Glucose Tolerance	Persons With Normal Glucose Tolerance
Estimated no. of capillaries per mm² muscle tissue	201 ± 49	238 ± 91*
Estimated no. of capillaries per muscle fiber	1.55 ± 0.62	1.70 ± 0.77
Estimated muscle fiber area per capillary (μm²)	3,467 ± 870	3,136 ± 1,086

NOTE. Data for capillarization are calculated from the number of extracellular endothelial nuclei in hematoxylin-stained sections of muscle tissue from musculus vastus lateralis. Muscle fiber area per capillary was calculated as the mean fiber area divided by the number of capillaries per fiber.

*$P < .05$.

†$P < .01$.

previous studies suggest that increased hepatic NEFA flux suppresses hepatic insulin extraction[48,49] and that hyperinsulinemia associated with obesity is partly accounted for by lower insulin clearance.[35,36] Our data support this view, since BMI, waist-to-hip ratio, and levels of triglycerides and NEFA were all found to correlate with the estimate of postload insulin clearance. Thus, the finding of increased postload insulin response in a glucose-intolerant population should not necessarily be interpreted as the presence of insulin resistance and normal early insulin release, as often is done.[4,5]

Our data indicate that glucose-intolerant persons may have normal insulin sensitivity, but that minor increments in body weight are associated with impairment in insulin-stimulated glucose uptake. Glucose intolerance is therefore not exclusively an insulin-resistant state as often postulated.[1,4-6,8,50] Previous reports did not adjusted for differences in BMI[1,50] or weight gain accompanying the development of glucose intolerance,[4] and elevated plasma insulin levels were frequently interpreted solely as a marker of insulin resistance.[4-6,8]

The association between BMI and measures of insulin kinetics and insulin action may be explained by the following hypothesis: enlargement of fat deposits leads to raised NEFA flux,[16] and thus, increased hepatic NEFA turnover. Increased hepatic NEFA turnover may reduce the hepatic insulin extraction[35,48,49] and lead to hyperinsulinemia.[51] Hyperinsulinemia may in turn downregulate the peripheral insulin receptors[52,53] and lead to decreased insulin sensitivity. Oxidative and nonoxidative NEFA turnover (NEFA reesterification) parallels plasma NEFA concentrations in healthy, as well as in insulin-resistant subjects.[17] Increased NEFA oxidation contributes to impaired insulin sensitivity for glucose disposal through the Randle cycle.[18] Recent studies on β-cell function have shown impairment of insulin secretion during long-term NEFA exposition.[54,55] Such a mechanism would further deteriorate the glycemic control in glucose-intolerant persons. In addition, increased NEFA availability is of importance for the development of thromboarterial disease. Elevated hepatic NEFA turnover increases triglyceride and very–low-density lipoprotein (VLDL)-cholesterol synthesis,[56] and perhaps also the hepatic synthesis of plasminogen activator inhibitor type 1 (PAI-1).[34]

In the present study, glucose-intolerant subjects had decreased insulin-induced NEFA suppression. They also had more type 2b fibers in their skeletal muscles compared with controls, as previously observed in subjects with non–insulin-dependent diabetes mellitus (NIDDM)[24] and obesity.[57] The percentage of type 2b muscle fibers found in the relatively lean glucose-intolerant subjects participating in the present study was lower than what others have observed in more obese persons.[24,57,58] This may explain why we did not find an association between the percentage of type 2b muscle fibers and insulin sensitivity for glucose disposal, as previously found in obesity[26,57] and NIDDM.[24] However, we did observe an association between the percentage of type 2b muscle fibers and insulin-induced NEFA suppression. The mechanism for this relationship is not clear. Impaired muscular NEFA uptake due to 2% higher proportion of type 2b muscle fibers, which do not metabolize fatty acids,[22] would account for about half of the group difference observed in NEFA availability during the clamp.

Glucose-intolerant persons differed from normoglycemic

controls in capillary density assessed as capillaries per square millimeter muscle tissue, but there were no associations between muscle capillarization and the metabolic variables. The degree of muscle capillarization may nevertheless influence glycemic control, as previously reported.[24,26,59] Measures of muscle capillarization are vulnerable to variations in capillary flow and capillary function, as well as the staining and counting method, and the fact that our study groups had a low degree of insulin resistance made it difficult to detect a relationship between muscle capillary density and insulin-stimulated glucose disposal.

In summary, we found that lean or moderately overweight glucose-intolerant persons had lower first-phase insulin release compared with glucose-tolerant persons, whereas second-phase insulin release and insulin sensitivity for glucose disposal did not differ, when standardizing for BMI. However, glucose-intolerant persons had decreased insulin clearance, increased insulin levels, and impaired insulin sensitivity to glucose disposal before standardizing for BMI. They also had an increased percentage of type 2b muscle fibers, which was associated with impaired NEFA suppression during hyperglycemic clamp. The present study suggests that lean or moderately overweight glucose-intolerant persons have impaired β-cell function for early insulin release, and an increased percentage of type 2b muscle fibers, which contributes to increased NEFA availability. We hypothesize that further elevation of NEFA availability associated with weight gain in persons with glucose intolerance plays an important role for the development of hyperinsulinemia and insulin resistance, and for deterioration of glycemic control.

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REFERENCES

1. Reaven GM, Hollenbeck CB, Chen YD: Relationship between glucose tolerance, insulin secretion, and insulin action in non-obese individuals with varying degrees of glucose tolerance. Diabetologia 32:52-55, 1989

2. Larsson H, Ahren B: Islet dysfunction in obese women with impaired glucose tolerance. Metabolism 45:502-509, 1996

3. Cook JT, Page RC, Levy JC, et al: Hyperglycaemic progression in subjects with impaired glucose tolerance: Association with decline in beta cell function. Diabet Med 10:321-326, 1993

4. Lillioja S, Mott DM, Howard BV, et al: Impaired glucose tolerance as a disorder of insulin action. Longitudinal and cross-sectional studies in Pima Indians. N Engl J Med 318:1217-1225, 1988

5. Modan M, Halkin H, Almog S, et al: Hyperinsulinemia. A link between hypertension, obesity and glucose tolerance. J Clin Invest 75:809-817, 1985

6. Charles MA, Pettitt DJ, Saad MF, et al: Development of impaired glucose tolerance with or without weight gain. Diabetes Care 16:593-596, 1993

7. Reaven GM: Banting lecture 1988. Role of insulin resistance in human disease. Diabetes 37:1595-1607, 1988

8. Saad MF, Knowler WC, Pettitt DJ, et al: Sequential changes in serum insulin concentration during development of non–insulin-dependent diabetes. Lancet 1:1356-1359, 1989

9. Felber JP, Golay A, Jequier E, et al: The metabolic consequences of long-term human obesity. Int J Obes 12:377-389, 1988

10. Cerasi E, Luft R: The plasma insulin response to glucose infusion in healthy subjects and in diabetes mellitus. Acta Endocrinol 55:278-304, 1967

11. Calles Escandon J, Robbins DC: Loss of early phase of insulin release in humans impairs glucose tolerance and blunts thermic effect of glucose. Diabetes 36:1167-1172, 1987

12. Kanatsuka A, Makino H, Sakurada M, et al: First-phase insulin response to glucose in nonobese or obese subjects with glucose intolerance: analysis by C-peptide secretion rate. Metabolism 37:878-884, 1988

13. Colman E, Katzel LI, Rogus E, et al: Weight loss reduces abdominal fat and improves insulin action in middle-aged and older men with impaired glucose tolerance. Metabolism 44:1502-1508, 1995

14. Cederholm J, Wibell L: What causes impaired glucose tolerance to deteriorate or normalize? Scand J Clin Lab Invest 52:491-496, 1992

15. Harris MI: Impaired glucose tolerance in the U.S. population. Diabetes Care 12:464-474, 1989

16. Groop LC, Saloranta C, Shank M, et al: The role of free fatty acid metabolism in the pathogenesis of insulin resistance in obesity and non–insulin-dependent diabetes mellitus. J Clin Endocrinol Metab 72:96-107, 1991

17. Groop LC, Bonadonna RC, DelPrato S, et al: Glucose and free fatty acid metabolism in non–insulin-dependent diabetes mellitus. Evidence for multiple sites of insulin resistance. J Clin Invest 84:205-213, 1989

18. Randle PJ, Garland PB, Hales CN, et al: The glucose fatty-acid cycle: Its role in insulin sensitivity and the metabolic disturbances of diabetes mellitus. Lancet 1:785-789, 1963

19. Jackson RA, Roshania RD, Hawa MI, et al: Impact of glucose ingestion on hepatic and peripheral glucose metabolism in man: An analysis based on simultaneous use of the forearm and double isotope techniques. J Clin Endocrinol Metab 63:541-549, 1986

20. Groop LC, Bonadonna RC, Simonson DC, et al: Effect of insulin on oxidative and nonoxidative pathways of free fatty acid metabolism in human obesity. Am J Physiol 263:E79-E84, 1992

21. James DE, Jenkins AB, Kraegen EW: Heterogeneity of insulin action in individual muscles in vivo: Euglycemic clamp studies in rats. Am J Physiol 248:E567-E574, 1985

22. Saltin B, Gollnick PD: Skeletal muscle adaptability: significance for metabolism and performance, in Peachey LD (ed): Handbook of physiology, Skeletal Muscle. Baltimore, MD, Williams & Wilkins, 1983, pp 555-631

23. Krotkiewski M, Bjorntorp P: Muscle tissue in obesity with different distribution of adipose tissue. Effects of physical training. Int J Obes 10:331-341, 1986

24. Marin P, Andersson B, Krotkiewski M, et al: Muscle fiber composition and capillary density in women and men with NIDDM. Diabetes Care 17:382-386, 1994

25. Krotkiewski M, Seidell JC, Bjorntorp P: Glucose tolerance and hyperinsulinaemia in obese women: Role of adipose tissue distribution, muscle fibre characteristics and androgens. J Intern Med 228:385-392, 1990

26. Lillioja S, Young AA, Culter CL, et al: Skeletal muscle capillary density and fiber type are possible determinants of in vivo insulin resistance in man. J Clin Invest 80:415-424, 1987

27. Bønaa KH, Arnesen E: Association between heart rate and atherogenic blood lipid fractions in a population. The Tromsø Study. Circulation 86:394-405, 1992

28. Bjorntorp P: Metabolic implications of body fat distribution. Diabetes Care 14:1132-1143, 1991

29. McGuire E, Helderman J, Tobin J, et al: Effect of arterial versus venous sampling on analysis of glucose kinetics in man. J Appl Physiol 41:565-573, 1976

30. Jorde R, Burhol PG, Schultz TB, et al: The effect of a 34-h fast on meal-induced rises in plasma GIP, serum insulin and blood glucose in man. Scand J Gastroenterol 16:109-112, 1981

31. Faber OK, Binder C: C-peptide response to glucagon: A test for the residual beta-cell function in diabetes mellitus. Diabetes 26:605-610, 1977

32. Birkeland KI, Torjesen PA, Eriksson J, et al: Hyperproinsulinemia of type 2 diabetes is not present before the development of hyperglycemia. Diabetes Care 17:1307-1310, 1994

33. Toft I, Bønaa KH, Ingebretsen OC, et al: Effects of n-3 polyunsaturated fatty acids on glucose homeostasis and blood pressure in essential hypertension. A randomized, controlled trial. Ann Intern Med 123:911-918, 1995

34. Toft I, Bønaa KH, Ingebretsen OC, et al: Gender differences in the relationships between plasma plasminogen activator inhibitor-1 activity and factors linked to the insulin restistance syndrome in essential hypertension. Arterioscler Thromb Vasc Biol 17:553-559, 1996

35. Meistas MT, Margolis S, Kowarski AA: Hyperinsulinemia of obesity is due to decreased clearance of insulin. Am J Physiol 245:E155-E159, 1983

36. Faber OK, Christensen K, Kehlet H, et al: Decreased insulin removal contributes to hyperinsulinemia in obesity. J Clin Endocrinol Metab 53:618-621, 1981

37. DeFronzo RA, Tobin J, Andres R: Glucose clamp technique. A method for quantifying insulin secretion and resistance. Am J Physiol 237:E214-222, 1979

38. Mitrakou A, Vuorinen Markkola H, Raptis G, et al: Simultaneous assessment of insulin secretion and insulin sensitivity using a hyperglycemia clamp. J Clin Endocrinol Metab 75:379-382, 1992

39. Bergstrom J: Muscle electrolytes in man. Scand J Clin Lab Invest 68:1-110, 1962 (suppl)

40. Round JM, Matthews Y, Jones DA: A quick, simple and reliable histochemical method for ATP-ase in human muscle preparations. Histochem J 12:707-710, 1980

41. Engel AG, Banker BQ: Alterations in the muscle fiber, in Engel AG, Franzini-Armstrong C (eds): Myology, vol 1. New York, NY, McGraw-Hill, 1994, p 838

42. Froes MM, Kristmundsdottir F, Mahon M, et al: Muscle morphometry in motor neuron disease. Neuropathol Appl Neurobiol 13:405-419, 1987

43. Engel WK, Cunningham GG: Rapid examination of muscle tissue: An improved trichrome stain method for fresh-frozen biopsy sections. Neurology 13:919-923, 1963

44. SAS Institute Inc: SAS/STAT Guide for Personal Computers, version 6 edition. Cary, NC, SAS Institute, 1987

45. Lerner RL, Porte DJ: Relationship between intravenous glucose loads, insulin responses and glucose disappearance rate. J Clin Endocrinol Metab 33:409-417, 1971

46. Mitrakou A, Kelley D, Mokan M, et al: Role of reduced suppression of glucose production and diminished early insulin release in impaired glucose tolerance. N Engl J Med 326:22-29, 1992

47. Marin P, Andersson B, Ottosson M, et al: The morphology and metabolism of intraabdominal adipose tissue in men. Metabolism 41:1242-1248, 1992

48. Peiris AN, Mueller RA, Struve MF, et al: Relationship of androgenic activity to splanchnic insulin metabolism and peripheral glucose utilization in premenopausal women. J Clin Endocrinol Metab 64:162-169, 1987

49. Peiris AN, Struve MF, Kissebah AH: Relationship of body fat distribution to the metabolic clearance of insulin in premenopausal women. Int J Obes 11:581-589, 1987

50. Eriksson J, Franssila Kallunki A, Ekstrand A, et al: Early metabolic defects in persons at increased risk for non–insulin-dependent diabetes mellitus. N Engl J Med 321:337-343, 1989

51. Kissebah AH, Peiris AN, Evans DJ: Mechanisms associating body fat distribution to glucose intolerance and diabetes mellitus: Window with a view. Acta Med Scand 723:79-89, 1988 (suppl)

52. Rizza RA, Mandarino LJ, Genest J, et al: Production of insulin resistance by hyperinsulinaemia in man. Diabetologia 28:70-75, 1985

53. Marangou AG, Weber KM, Boston RC, et al: Metabolic consequences of prolonged hyperinsulinemia in humans. Evidence for induction of insulin insensitivity. Diabetes 35:1383-1389, 1986

54. Paolisso G, Gambardella A, Amato L, et al: Opposite effects of short- and long-term fatty acid infusion on insulin secretion in healthy subjects. Diabetologia 38:1295-1299, 1995

55. Zhou YP, Grill V: Long term exposure to fatty acids and ketones inhibits B-cell functions in human pancreatic islets of Langerhans. J Clin Endocrinol Metab 80:1584-1590, 1995

56. Byrne CD, Brindle NP, Wang TW, et al: Interaction of non-esterified fatty acid and insulin in control of triacylglycerol secretion by Hep G2 cells. Biochem J 280:99-104, 1991

57. Krotkiewski M, Bylund Fallenius AC, Holm J, et al: Relationship between muscle morphology and metabolism in obese women: The effects of long-term physical training. Eur J Clin Invest 13:5-12, 1983

58. Wade AJ, Marbut MM, Round JM: Muscle fibre type and aetiology of obesity. Lancet 335:805-808, 1990

59. Lithell H, Lindgarde F, Hellsing K, et al: Body weight, skeletal muscle morphology, and enzyme activities in relation to fasting serum insulin concentration and glucose tolerance in 48-year-old men. Diabetes 30:19-25, 1981