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Oxidative and nonoxidative glucose disposal in elderly vs younger men with similar and smaller body mass indices and waist circumferences☆

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Abstract

Defects in oxidative and nonoxidative glucose metabolism may be involved in the insulin resistance of aging, possibly linked to a central redistribution of body fat. By hyperinsulinemic-euglycemic clamps with whole-body indirect calorimetry, we assessed the contributions of oxidative and nonoxidative glucose disposal to insulin action in 12 elderly persons and 2 groups of younger subjects (14 in each) who had participated in a large population study. Subjects from Young-1 were individually matched to the elderly persons by body mass index and had similar waist circumferences, whereas subjects from Young-2 had a body mass index typical of their age group in the population study and smaller waist measurements. In the combined sample, we also considered possible determinants, related to age and central fat, of flux through these metabolic pathways. The elderly persons had lower nonoxidative glucose disposal compared with the men in Young-2 (P = .0450 by analysis of variance), whereas glucose oxidation did not differ between the groups. Glucose oxidation correlated negatively with waist circumferences, triglycerides, and alanine aminotransferase and positively with total testosterone and sex hormone–binding globulin. Nonoxidative glucose metabolism correlated inversely with waist circumferences, triglycerides, and free fatty acids and positively with maximum O_2 consumption and total testosterone. In the best regression models, alanine aminotransferase and triglycerides were negatively associated with glucose oxidation (model R^2 = 39%), whereas lower baseline free fatty acids and higher maximum O_2 consumption and sex hormone–binding globulin predicted enhanced nonoxidative glucose metabolism (model R^2 = 47%). These results substantiate that measures to avert abdominal adiposity may prevent insulin resistance and its related metabolic derangements in elderly persons.

1. Introduction

Insulin action on glucose disposal deteriorates with aging, possibly because of age-related augmententations in adiposity, particularly central adiposity [1-4]. Defects in oxidative or nonoxidative glucose metabolism could be involved when insulin action declines in elderly people, and, in fact, both of these metabolic pathways have been indicted [5-8]. Conceivably, such deficiencies could be caused by increased body fat because greater adiposity is recognized to impair glucose disposal through both routes [9].

However, whereas obesity appears to induce insulin resistance by interference of free fatty acids (FFAs) and intramyocellular lipids with insulin signaling [10,11], the aging process per se may diminish insulin action through disparate mechanisms (eg, related to amplified oxidative stress) [12]. In addition, other distinctive features of aging, also coupled with body fat, could influence the extent of flow through the oxidative and nonoxidative glucose metabolic pathways. Such confounding factors include a central accumulation of fat, reduced physical fitness, altered androgen status, and changes in serum lipids and liver fat and have only occasionally been considered.

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In a population-based euglycemic clamp study, we have previously reported that elderly and younger men matched by body mass index (BMI) and with comparable waist circumferences had similar glucose disposal rates and insulin sensitivity indices [4]. However, young individuals with a BMI typical for their age group and smaller waist circumferences had enhanced insulin action. For the present study, we concomitantly determined in the same groups glucose oxidation and nonoxidative disposal by whole-body indirect calorimetry to address the impacts of body fat vs age on the contributions of these 2 pathways of glucose metabolism. In addition, by correlation and regression analysis of data in the combined sample, we considered the influences of age, central fat, maximum O_2 consumption ($\dot{V}O_2$max), androgen status, lipids, and alanine aminotransferase (ALT) on these routes.

2. Materials and methods

2.1. The Tromsø study

The participants were selected and matched using the database of the fourth survey of the Tromsø Study [13]; their health status was assessed at a screening visit as previously reported [4]. All subjects were healthy, without evident cardiovascular, pulmonary, hepatic, renal, or metabolic disease at the time of inclusion and were nonsmokers and weight stable.

Briefly, to 15 elderly participants aged 71 to 77 years, we individually matched 15 younger participants aged 31 to 33 years (Young-1) by BMI. Another group of young persons (Young-2) consisted of 15 participants also aged 31 to 33 years but with a BMI representative of the age group in the population study. The present study consists of subjects in whom valid indirect calorimetry data were obtained. The Regional Board of Research Ethics approved the study and all participants gave written informed consent to participate.

2.2. Research design

The participants were tested on 3 separate days. All underwent a bicycle ergometer test to determine $\dot{V}O_2$max, followed by an oral glucose tolerance test and a hyperinsulinemic-euglycemic clamp in combination with indirect whole-body calorimetry (see below).

2.3. Maximum O_2 consumption

Maximum O_2 consumption was determined by a metabolic measurement cart (SensorMedics 2900, SensorMedics Corp, Yorba Linda, Calif) during exertion on an electronically braked bicycle ergometer (Ergomed 840, Siemens, Erlangen, Germany) until subjective exhaustion, as previously reported [4].

2.4. Anthropometric measurements

We determined the subjects' weight to the nearest 100 g on an electronic scale and height to the nearest centimeter on a wall-mounted stadiometer and calculated the BMI as the weight divided by the square of the height. We measured the waist circumference as the minimal circumference of the abdomen at the end of a normal expiration and the hip circumference as the circumference of the buttocks at the maximal gluteal protuberance and calculated the waist-to-hip ratio (WHR).

2.5. Body composition

Percent body fat was determined by near-infrared interactance using a Futrex-5000 Body Composition Analyzer (Futrex, Inc, Gaithersburg, Md) at the biceps site of the dominant arm. Body fat mass was calculated by multiplying percent body fat by the body weight and fat-free mass (FFM) by subtracting the fat mass from the body weight.

2.6. Oral glucose tolerance test

The subjects received 1 g dextrose/kg body weight, maximum 75 g. Arterialized venous blood samples were taken at −30, 0, 10, 20, 30, 60, 90, and 120 minutes. Glucose responses were classified according to the 1999 World Health Organization criteria [14].

2.7. Euglycemic clamp

Hyperinsulinemic-euglycemic clamps were performed as described previously [4]. Briefly, a catheter was placed in a left antecubital vein and a 20 μCi priming dose of sterile, pyrogen-free 3-^{3}H glucose (Amersham, Buckinghamshire, UK) was given at −150 minutes, followed by a 0.20 μCi/min infusion lasting 5½ hours. An insulin infusion [rate, 0.4 mU/(kg · min)] was started at 0 minutes and continued for 3 hours. The insulin infusion rates were targeted at plasma insulin concentrations near the median effective dose (ED_{50}) for the suppression of endogenous glucose output (EGO) and lipolysis [4]. Plasma glucose concentrations were determined at 5-minute intervals and maintained at 5 mmol/L by a variable infusion of 25% glucose. To minimize rapid dilution of the labeled glucose pool, 0.02 μCi/mL 3-^{3}H glucose was added to the glucose infusion (hot-GINF) [15].

2.8. Indirect calorimetry

Whole-body indirect calorimetry [16] was used to determine O_2 consumption and CO_2 production during the last 30 minutes of the basal and hyperinsulinemic-euglycemic clamp periods to estimate net rates of carbohydrate and lipid oxidation. A metabolic measurement cart (SensorMedics 2900, SensorMedics Corp) with a computerized open-circuit system was used to measure gas exchange through a transparent plastic canopy, which was made airtight at the neck. Flow was measured by the air dilution method, CO_2 concentration by a conventional infrared detector, and O_2 concentration by a zirconium analyzer. Before the study, the subjects were acquainted with the procedure by sham demonstrations. Measurements were

obtained at a mean of 17 ± 0.5 minutes (SEM) in the basal state and during hyperinsulinemia.

2.9. Substrate oxidation

Net glucose and lipid oxidation rates were calculated from indirect calorimetric measurements using a constant value for urinary nitrogen excretion [17]. The standard equations of Frayn [18] were used:

$$\text{Glucose oxidation (g/min)} = 4.55\ \dot{V}_{CO_2} - 3.21\ \dot{V}_{O_2} - 2.87N$$

$$\text{Lipid oxidation (g/min)} = 1.67\ \dot{V}_{O_2} - 1.67\ \dot{V}_{CO_2} - 1.92N$$

where $\dot{V}_{O_2}$ and $\dot{V}_{CO_2}$ are the consumption and production rates of O_2 and CO_2, respectively, and N is an estimated urinary nitrogen excretion rate of 0.009 g/min [17]. A negative value for lipid oxidation was observed in one person in each group during hyperinsulinemia and was assumed to be zero.

2.10. Analytical methods

Plasma glucose concentrations were measured bedside on a YSI glucose analyzer (2300 STAT PLUS, Yellow Springs Instrument Co, Yellow Springs, Ohio). Plasma insulin [4] and serum total testosterone (RSL, ICN Biomedicals Inc, Costa Mesca, Calif) were measured by radioimmunoassay, serum FFA levels by spectrophotometry (kit from Wako Chemicals GmbH, Neuss, Germany), and serum sex hormone–binding globulin (SHBG) (Farmos Group Ltd, Oulonsalo, Finland) with an immunometric method. Specific activities in 3-^{3}H glucose tracer infusions and EDTA plasma samples were determined by liquid scintillation counting, as previously detailed [4]. Serum triglyceride levels and ALT activities were analyzed on a Hitachi 917 Automatic Analyzer (Hitachi Ltd, Tokyo, Japan) with reagents from Roche Diagnostics Corp (Mannheim, Germany).

2.11. Calculations

Baseline levels of plasma glucose, insulin, and serum FFAs were calculated as the mean of the concentrations at −60, −30, and 0 minutes. Plasma glucose, insulin, and serum FFAs during steady-state hyperinsulinemia were taken as the average of the values at 120, 150, and 180 minutes. The glucose infusion rate was determined as the mean of the glucose infusion rates at 120, 150, and 180 minutes. Turnover data in the basal state and during steady-state hyperinsulinemia were calculated from the glucose-specific activities from −60 to 0 and 120 to 180 minutes and negative values for EGO were assumed to be zero, as previously described [4]. Nonoxidative glucose disposal rate was calculated as total glucose disposal rate minus glucose oxidation rate. Negative values for nonoxidative glucose disposal were encountered in 4 persons (1 elderly subject, 3 subjects from Young-2) in the basal state and in 2 persons (1 elderly subject, 1 subjects from Young-1) during hyperinsulinemia. These values were interpreted as zero. The insulin sensitivity index was calculated as the total glucose disposal rate between 120 and 180 minutes divided by the insulin concentration over the same period.

2.12. Statistics

The primary study [4] was designed to detect a 20% difference in total glucose disposal with 90% power, with 12 participants in the groups. To allow for missing data, we recruited 15 in each. The oral glucose tolerance test results were discarded for one person in Young-2 because of exertion before the test. Serum triglyceride measurements were missing for one elderly subject and basal FFA measurements for one individual in Young-2. Indirect calorimetry results either at baseline or during hyperinsulinemia were obtained in 13 elderly participants, in 15 persons from Young-1, and in 14 subjects from Young-2. Baseline results were discarded in one elderly person because of strain during the procedure and in one subject in each group because of technical difficulties. Data during hyperinsulinemia were rejected in one elderly and one young person (Young-1) owing to strain, in one elderly participant because of technical problems, and in one subject from Young-2 owing to loss of tracer sample. Indirect calorimetry was not performed in one elderly participant as a result of distress on starting the procedure. Measurements under both conditions were obtained in 11, 13, and 13 participants, respectively.

The slopes of the associations between glucose disposal and FFM did not differ among the groups and the y-intercepts were not statistically different from zero, and turnover data were expressed per kilogram FFM. The FFA and EGO values during hyperinsulinemia were highly skewed, and group differences were assessed by Kruskal-Wallis tests. For the other variables, differences were evaluated with analysis of variance and multiple comparisons were performed with Bonferroni t tests. The univariate associations of total, oxidative, and nonoxidative glucose disposal rates per kilogram FFM with continuous variables were analyzed with Spearman rank correlation. Backward-selection multiple regression was used to discern the best models to predict the same dependent variables. First, age, waist circumferences, steady-state insulin concentrations, $\dot{V}_{O_2}$max, triglycerides, total testosterone, SHBG, and levels of FFAs in the basal state and during hyperinsulinemia were considered as independent variables for total glucose disposal, based on results from previous studies [6,19,20]. Next, the ALT activities were also taken into account [21]. The best models to predict oxidative and nonoxidative glucose disposal were then obtained with all these independent variables in the full model. The waist circumference was chosen rather than the waist-to-hip ratio because of its stronger correlation with insulin action [1,4]. Data in the text and tables are given as mean ± SEM and

$P < .05$ was considered significant. The data were analyzed with the SAS software package (SAS Institute Inc, Cary, NC).

3. Results

3.1. Subject characteristics

Body fat was highest in the elderly persons and lowest in subjects from Young-2, but the differences between the groups were not significant. The FFM was higher in the subjects from Young-1 compared with those from the elderly group and Young-2. The waist circumferences were significantly lower and the total testosterone levels were higher in the subjects from Young-2 than in those from the elderly group and Young-1, who had similar measurements of these variables. The SHBG, systolic blood pressures, 2-hour glucose, and area under the glucose curve were higher in the elderly persons compared with the subjects from the 2 younger groups, in whom the levels of these variables did not differ. The $\dot{V}O_2$max differed between all groups and was lowest in the elderly subjects. The ALT levels were reduced in the elderly persons compared with subjects from Young-1 (Table 1). Two elderly persons and 4 individuals from Young-1 had first-degree relatives with type 2 diabetes. Four elderly subjects had a 2-hour plasma glucose concentration between 8.9 and 12.2 mmol/L; all of them had a fasting plasma glucose concentration below 7.0 mmol/L. One elderly person had a fasting plasma glucose concentration between 6.1 and 7.0 mmol/L together with a 2-hour value below 8.9 mmol/L (impaired fasting glycemia). None of the younger control subjects had glucose abnormalities (Table 1).

3.2. Metabolic data

3.2.1. Basal state

The FFA levels were highest in the elderly group ($P = .0266$ for between-group differences). Otherwise, the metabolic variables did not differ significantly between the groups. In the elderly persons, oxidative and nonoxidative glucose metabolism contributed equally to glucose disposal, whereas in the subjects from each of the younger groups, glucose oxidation constituted approximately two thirds of total glucose disposal (Table 2).

3.2.2. Hyperinsulinemia

The plasma insulin levels were higher in the elderly group than in Young-2 ($P = .0065$ for between-group differences), but there were no significant differences between the elderly group and Young-1 or between Young-1 and Young-2. Glucose disposal rates did not differ between the elderly and Young-1 but were higher in Young-2 ($P = .0111$ for between-group differences). The levels of nonoxidative glucose disposal were lower in the elderly group compared with Young-2, whereas intermediate values were observed in Young-1 ($P = .0450$ for between-group differences). Glucose oxidation rates did not differ significantly between the groups. In the elderly group, glucose oxidation increased during hyperinsulinemia to contribute by approximately three fourths to glucose disposal. However, in both younger groups, the fraction of total glucose disposal represented by

Table 1
Subject characteristics

	Elderly group		Young-1		Young-2		P (analysis of variance)
	n	Mean	n	Mean	n	Mean	
Age (y)	13	72.6 ± 0.5	15	32.0 ± 0.1	14	32.1 ± 0.1	Selection variable
BMI (kg/m^2)	13	24.8 ± 0.5	15	25.5 ± 0.6	14	23.8 ± 0.5	Matching variable
Body weight (kg)	13	77.0 ± 1.7[a]	15	84.8 ± 2.1[b]	14	75.1 ± 1.6	.0010
Body fat (%)	13	17.8 ± 1.5	15	13.4 ± 1.7	14	12.8 ± 1.8	.0981
Body fat (kg)	13	13.9 ± 1.3	15	11.5 ± 1.5	14	9.7 ± 1.4	.1493
FFM (kg)	13	63.4 ± 1.2[a]	15	73.6 ± 2.1[b]	14	65.8 ± 1.7	.0006
Waist circumference (cm)	13	94.3 ± 1.9	15	91.3 ± 1.7[b]	14	84.1 ± 1.6[c]	.0006
Waist-to-hip ratio	13	0.95 ± 0.01[a]	15	0.89 ± 0.01	14	0.86 ± 0.01[c]	.0001
Systolic blood pressure (mm Hg)	13	136 ± 3[a]	15	125 ± 3	14	126 ± 3[c]	.0108
$\dot{V}O_2$max [mL/(kg · min)]	13	26.6 ± 1.1[a]	15	39.5 ± 1.5[b]	14	44.6 ± 1.6[c]	.0001
Serum triglycerides (mmol/L)	12	1.2 ± 0.1	15	1.3 ± 0.1[b]	14	0.9 ± 0.1	.0234
Serum ALT (U/L)	13	24 ± 2[a]	15	36 ± 3	14	29 ± 3	.0393
Serum total testosterone (nmol/L)	13	13.6 ± 1.6	15	15.6 ± 1.0[b]	14	21.7 ± 1.6[c]	.0007
Serum SHBG (nmol/L)	13	39 ± 4[a]	15	18 ± 2	14	25 ± 2[c]	.0001
Fasting plasma glucose (mmol/L)	13	5.7 ± 0.1	15	5.3 ± 0.2	13	5.3 ± 0.1	.0721
Two-hour plasma glucose (mmol/L)	13	8.4 ± 0.4[a]	15	6.5 ± 0.4	13	6.0 ± 0.3[c]	.0003
Area under the glucose curve (mmol · min/L)	13	1081 ± 55[a]	15	890 ± 20	13	905 ± 26[c]	.0008

Persons with indirect calorimetry results.

Young-1, young persons matched to the elderly patients according to BMI; Young-2, young persons with BMI representative of all males of this age group in the population survey. Differences significant at the .05 level by Bonferroni t tests.

[a] Elderly vs Young-1.

[b] Young-1 vs Young-2.

[c] Elderly vs Young-2.

Table 2
Metabolic data

	Elderly group		Young-1		Young-2		*P*
	n	Mean	n	Mean	n	Mean	
Basal state							
Plasma glucose (mmol/L)	11	5.60 ± 0.20	14	5.48 ± 0.07	13	5.50 ± 0.08	.7709
Plasma insulin (pmol/L)	11	37 ± 4	14	49 ± 6	13	38 ± 7	.3187
Serum FFAs (mmol/L)	11	0.44 ± 0.04	14	0.30 ± 0.03	12	0.30 ± 0.04	.0266
Total glucose disposal rate [μmol/(kg_{FFM} · min)]	11	15.2 ± 0.7	14	14.2 ± 0.8	13	14.6 ± 0.8	.6952
Glucose oxidation rate [μmol/(kg_{FFM} · min)]	11	8.1 ± 1.1	14	9.4 ± 0.9	13	11.0 ± 1.0	.1682
Nonoxidative glucose disposal rate [μmol/(kg_{FFM} · min)]	11	7.1 ± 0.9	14	4.8 ± 1.0	13	4.3 ± 0.9	.1142
Glucose oxidation (% of total glucose disposal)	11	53 ± 7	14	67 ± 6	13	72 ± 6	.0899
Nonoxidative glucose disposal (% of total glucose disposal)	11	47 ± 7	14	33 ± 6	13	28 ± 6	.0899
Lipid oxidation rate [μmol/(kg_{FFM} · min)]	11	0.86 ± 0.09	14	0.91 ± 0.07	13	0.82 ± 0.11	.7692
Respiratory quotient	11	0.83 ± 0.01	14	0.83 ± 0.01	13	0.85 ± 0.01	.4247
Hyperinsulinemia							
Plasma glucose (mmol/L)	12	5.08 ± 0.03	14	5.02 ± 0.02	14	5.03 ± 0.04	.3216
Plasma insulin (pmol/L)	12	216 ± 13	14	185 ± 9	14	165 ± 9[b]	.0065
Serum FFAs (mmol/L)	12	0.04 ± 0.01	14	0.04 ± 0.01	14	0.02 ± 0.01	.4532[a]
Glucose infusion rate [μmol/(kg_{FFM} · min)]	12	19.0 ± 2.1	14	19.1 ± 3.2	14	28.4 ± 3.3	.0487
EGO [μmol/(kg_{FFM} · min)]	12	2.2 ± 0.9	14	3.3 ± 0.9	14	1.8 ± 0.7	.4820[a]
Total glucose disposal rate [μmol/(kg_{FFM} · min)]	12	19.8 ± 1.0	14	20.5 ± 1.8[c]	14	26.9 ± 2.1[b]	.0111
Glucose oxidation rate [μmol/(kg_{FFM} · min)]	12	15.3 ± 0.8	14	12.7 ± 1.5	14	16.3 ± 1.0	.0888
Nonoxidative glucose disposal rate [μmol/(kg_{FFM} · min)]	12	4.8 ± 1.0	14	7.8 ± 1.3	14	10.7 ± 2.1[b]	.0450
Glucose oxidation (% of total glucose disposal)	12	77 ± 4	14	62 ± 5	14	64 ± 5	.0767
Nonoxidative glucose disposal (% of total glucose disposal)	12	23 ± 4	14	38 ± 5	14	36 ± 5	.0767
Lipid oxidation rate [μmol/(kg_{FFM} · min)]	12	0.31 ± 0.06[d]	14	0.63 ± 0.10	14	0.42 ± 0.09	.0461
Respiratory quotient	12	0.91 ± 0.01	14	0.87 ± 0.02	14	0.91 ± 0.01	.0920
Insulin sensitivity index[e]	12	0.10 ± 0.01	14	0.12 ± 0.01[c]	14	0.17 ± 0.02[b]	.0018

Subjects with indirect calorimetry results in the basal state and during hyperinsulinemia, respectively.
Differences significant at the .05 level by Bonferroni *t* tests:

[a] *P* value by Kruskal-Wallis test, all other *P* values by analysis of variance.
[b] Elderly vs Young-1.
[c] Young-1 vs Young-2.
[d] Elderly vs Young-2.
[e] Insulin sensitivity index is steady-state total glucose disposal rate divided by steady-state plasma insulin.

glucose oxidation remained similar to that observed in the basal state. Lipid oxidation was lower in the elderly group compared with Young-1, whereas the respiratory quotient did not differ between the groups (Table 2).

3.2.3. Correlation and multiple regression analysis in the total sample

Higher waist circumferences and triglyceride levels were negatively associated with total glucose disposal, glucose oxidation, and nonoxidative glucose metabolism, and the correlations were stronger than those with total body fat. Free fatty acids in the basal state and during hyperinsulinemia both correlated inversely with total and nonoxidative glucose metabolism, whereas ALT correlated negatively with total and oxidative glucose disposal. Higher total testosterone was associated with enhanced total, oxidative, and nonoxidative glucose metabolism, whereas SHBG correlated positively with glucose oxidation and higher $\dot{V}O_2$max correlated with enhanced total and nonoxidative glucose metabolism (Tables 3 and 4).

In the best model identified to predict total glucose disposal, the waist circumferences, triglycerides, and basal FFA levels were negatively associated with total glucose disposal and the SHBG concentrations were positively associated with total glucose disposal, provided that the

Table 3
Spearman correlation coefficients of insulin-stimulated total, oxidative, and nonoxidative glucose disposal with selected variables

	n	Total glucose disposal	Glucose oxidation	Nonoxidative glucose disposal
Waist circumference (cm)	40	−0.66*	−0.38***	−0.46**
Waist-to-hip ratio	40	−0.46**	−0.06	−0.45**
Body fat (%)	40	−0.44**	−0.28	−0.24
Body fat (kg)	40	−0.49*	−0.34****	−0.24
$FFAs_{basal\ state}$ (mmol/L)	39	−0.47**	−0.04	−0.50*
$FFAs_{hyperinsulinemia}$ (mmol/L)	40	−0.49*	−0.22	−0.37***
Serum triglycerides (mmol/L)	39	−0.62*	−0.43**	−0.36****
ALT (U/L)	40	−0.43**	−0.52*	−0.18
$\dot{V}O_2$max [mL/(kg · min)]	40	0.44**	0.03	0.45**
Total testosterone (nmol/L)	40	0.51*	0.31****	0.39***
SHBG (nmol/L)	40	0.28	0.37***	0.05

* $P < .002$.
** $.002 < P < .01$.
*** $.01 < P < .02$.
**** $.02 < P < .05$.

Table 4
Results of backward-selection multiple regression analysis for the effects of selected variables on insulin-stimulated total, oxidative, and nonoxidative glucose disposal

Dependent variable	Independent variables	β	SEM	F	*P*
Total glucose disposal					
Model 1 (R^2 = 0.62)	Waist	−.33202424	0.11363677	8.54	.0062
	Triglycerides	−4.16691689	1.81889425	5.25	.0285
	SHBG	.14125752	0.06503312	4.72	.0371
	$FFAs_{basal\ state}$	−18.56535880	6.51232850	8.13	.0075
Model 2 (with ALT, R^2 = 0.65)	Age	−.16222001	0.05936044	7.47	.0101
	ALT	−.14947461	0.06717622	4.95	.0332
	Triglycerides	−4.51552187	1.72654858	6.84	.0135
	SHBG	.24689255	0.08334538	8.78	.0057
	$FFAs_{basal\ state}$	−18.70923111	6.34754959	8.69	.0059
Glucose oxidation (model R^2 = 0.39)	ALT	−.16046189	0.04724424	11.54	.0017
	Triglycerides	−3.23702809	1.27880693	6.41	.0160
Nonoxidative glucose disposal (model R^2 = 0.47)	$\dot{V}O_2$max	.28922266	0.11107652	6.78	.0136
	SHBG	.16502337	0.06270259	6.93	.0127
	$FFAs_{basal\ state}$	−17.65790986	6.82755455	6.69	.0142

Age, waist circumferences, steady-state insulin concentrations, $\dot{V}O_2$max, serum triglycerides, total testosterone, SHBG, and FFAs in the basal state and during hyperinsulinemia considered as independent variables in model 1. Alanine aminotransferase also considered in 3 other full models.

ALT activities were not considered (model 1, R^2 = 62%). However, when the ALT activities were included in the full model, age and ALT levels replaced the waist circumferences as negative predictors of total glucose disposal (model 2, R^2 = 65%). In the best model to predict glucose oxidation (model R^2 = 39%), the ALT and triglyceride levels were negatively associated with this route of glucose disposal, and the triglycerides exerted the most influence. In the best model for nonoxidative glucose disposal (model R^2 = 47%), basal FFA levels were negative and $\dot{V}O_2$max and SHBG concentrations were positive predictors of this pathway, and the 3 variables had a similar impact.

4. Discussion

In a study with an original design, we have previously reported that older and younger men matched by BMI had identical insulin-stimulated glucose disposal rates, whereas young men with BMI typical of their age group had better insulin action [4]. The present results obtained during the same euglycemic clamps strongly indicate that different rates of nonoxidative glucose disposal were the main cause of the discrepancy in insulin action between the groups. Older and younger persons with similar BMI and waist circumferences had rates of nonoxidative glucose disposal that did not differ significantly across an age span of 40 years, although the rates observed were somewhat lower in the elderly group. In contrast, when compared with the young participants who were leaner, the older subjects had lower rates of nonoxidative glucose disposal. This could sustain a role of body fat in the decline in nonoxidative glucose disposal seen with age.

Although indices of general obesity determine the deterioration of insulin action seen with aging [3], markers of abdominal fat seem to have a greater impact on this decline [1-4,22]. The present results indicate a negative influence of a central fat distribution, stronger than that of total body fat, on oxidative as well as nonoxidative insulin-stimulated glucose disposal, which is in line with previous findings [19].

A correlation study [8] has formerly alleged impaired nonoxidative glucose metabolism as the main cause of the diminished insulin-stimulated glucose disposals seen with aging in men. At higher insulin concentrations than those in our study, Boden et al [7] observed that both oxidative and nonoxidative glucose disposal rates tended to be lower in elderly than in young men matched for weight and height. Yet, over a range of insulin doses, Bonadonna et al [6] found that nonoxidative glucose disposal was similar in younger and older persons matched by weight and BMI, whereas glucose oxidation was lower in the elderly persons. Another study [5] also concluded that the insulin resistance of aging was caused by decreased glucose oxidation.

Limited numbers of subjects, selected and matched by different criteria, may account for discrepancies between studies. To our knowledge, the current study is the largest to consider the impact of aging on oxidative and nonoxidative insulin-stimulated glucose disposal, and its design is unusual.

Different insulin doses may also contribute to divergent results. Although the nonoxidative pathway has a higher capacity for glucose metabolism, its sensitivity to insulin is lower than that for the oxidative pathway (ED_{50} ~200 and ~100 pmol/L, respectively) [23]. Accordingly, the insulin levels in this study were closer to the ED_{50} for the nonoxidative than oxidative pathway, which could imply that a difference in nonoxidative glucose metabolism would be more readily detected.

Although we chose insulin doses to achieve steady-state insulin concentrations of around ED_{50} for inhibition of lipolysis, the levels that we actually attained were somewhat higher, which limits the precision of the FFA results during hyperinsulinemia. Still, negative relationships of not only

FFA levels but also of FFA turnover rates with both oxidative and nonoxidative glucose metabolism were observed by Bonadonna et al [6] in a group of elderly and young individuals. Taken together, our results and previous observations [6] would suggest a detrimental effect of higher FFA concentrations (perhaps secondary to augmented abdominal fat [24,25]) on insulin-stimulated nonoxidative and oxidative glucose disposal.

Raised serum ALT [26], even within the reference range [27], is a marker of liver fat. Although increased ALT and liver fat are linked to central fat [21,28], the prevalence of elevated ALT activity decreases with advancing age [28], which could explain why age only entered the prediction model for total glucose disposal when the ALT levels were considered.

A novel finding of the present study is that ALT activities, together with a central distribution of body fat and higher triglycerides [20], predict lower total and oxidative glucose disposal. Previously, Kelley et al [29] found that in persons with type 2 diabetes, individuals with fatty liver had lower rates of nonoxidative glucose disposal than the subjects with normal liver attenuation, who also had less abdominal fat and lower FFA levels.

Ectopic accumulation of fat [30] in skeletal muscle and liver is strongly related with impaired insulin action. Increased amounts of central and peripheral fat lead to increased flux of FFA to the liver and skeletal muscle [10,11,31,32], which may be stored as triglycerides. Although a great deal is known about the relationships of FFA and intramyocellular lipids with reduced insulin signaling and insulin resistance [10,11], the precise mechanisms by which impaired insulin action is linked to hepatic fat are less clear [31].

Maximum O_2 consumption generally decreases with age, although physical training may modulate this decline [33]. However, exercise training, with or without weight loss, also reduces abdominal fat [34]. As reported previously [20], we observed that the $\dot{V}O_2$max levels correlated positively with nonoxidative glucose disposal but not with glucose oxidation. In addition, $\dot{V}O_2$max levels entered the best model to predict nonoxidative glucose metabolism. This points to a counteractive effect of exercise training and higher $\dot{V}O_2$max on the deterioration in nonoxidative glucose disposal observed in aging, which could occur in parallel with a reduction in central fat.

Testosterone levels decline with aging [35] and impaired androgen status in men is related to abdominal obesity [32]. Previously, in men with type 2 diabetes, Birkeland et al [36] have demonstrated positive correlations of both SHBG and total testosterone with insulin action, still significant after adjustments for obesity and body fat distribution. In normoglycemic men, Haffner et al [19] have reported positive relationships of both total and free testosterone and SHBG concentrations with total and nonoxidative glucose disposal, and we have observed similar associations with SHBG. These findings indicate close links between testosterone and SHBG levels and total and nonoxidative glucose metabolism in men.

Our data should be interpreted with some caution because a substantial number of analyses were performed with several intercorrelated variables. Still, complementary parts of our work support a view that increased total fat and central fat, as well as other body changes related to adiposity and aging (but not chronological age per se), cause insulin resistance.

A concern is that we determined body fat by near-infrared interactance, which has been found to systematically underestimate body fat, especially with increased adiposity [37]. A greater underestimation of body fat caused by a redistribution of fat from peripheral to central regions in the elderly and younger persons matched to them by BMI could thus have influenced our results. However, similar methodological problems, caused by aging and body fat redistribution, apply to several alternative techniques to determine body composition [38].

A shortcoming of indirect calorimetry is that nonoxidative glucose metabolism is not measured directly but is calculated by subtracting the value for glucose oxidation from total glucose disposal. It therefore depends on the correct estimation of both. Of note is that we used a constant value [17] to approximate urinary nitrogen excretion. However, any consequent effect on the results was probably very small for carbohydrate oxidation during hyperinsulinemia [39] and for the elderly participants who were healthy [40].

In summary, our data in men indicate that diminished rates of insulin-stimulated nonoxidative glucose metabolism are the main cause of the lower insulin action seen in elderly persons and suggest that age-related adiposity plays a role in this decline. Central fat was linked to impaired oxidative as well as nonoxidative glucose metabolism. In regression analysis, lower $\dot{V}O_2$max and SHBG together with higher basal FFA levels best predicted lower insulin-stimulated nonoxidative glucose metabolism, whereas higher ALT and triglyceride concentrations were associated with diminished glucose oxidation. These findings point toward a relationship of both oxidative and nonoxidative glucose metabolism with metabolic mechanisms connected to central fat.

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