

Aging Per Se Does Not Influence Glucose Homeostasis

In vivo and in vitro evidence

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OBJECTIVE — To assess the effect of age on glucose metabolism by examining 1) glucose metabolism in young and middle-aged subjects when total or regional adiposity is taken into account and 2) in vitro glucose transport in adipose tissue explants from young and middle-aged women paired for total and abdominal adiposity.

RESEARCH DESIGN AND METHODS — Study 1: body composition, subcutaneous abdominal and visceral adipose tissue areas, and fasting and oral glucose-stimulated glucose and insulin were measured in 84 young and 81 middle-aged men and in 110 young and 91 middle-aged women. Study 2: glucose uptake in subcutaneous abdominal and visceral adipose tissue explants were measured in eight young and eight middle-aged women.

RESULTS — Study 1: young and middle-aged men showed similar subcutaneous abdominal tissue area, whereas fat mass and visceral adipose tissue were greater in middle-aged than in young men ($P < 0.01$). Fat mass and subcutaneous and visceral adipose tissue areas were greater in middle-aged as compared with young women ($P < 0.01$). Fasting plasma glucose and the glucose response to an oral glucose tolerance test were significantly higher in middle-aged than in young men and women ($P < 0.001$). Statistical control for visceral adipose tissue area eliminated the difference seen in glucose response in men and women. Study 2: glucose transport in subcutaneous and omental adipose tissue did not differ between young and middle-aged women.

CONCLUSIONS — 1) Visceral obesity, more than age per se, correlates with glucose intolerance in middle-aged subjects; 2) aging does not influence in vitro adipose tissue glucose uptake.

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Adiposity has a potent effect on insulin sensitivity (1). Insulin resistance in obesity is commonly believed to manifest by decreased insulin-stimulated glucose transport in skeletal muscle and by impaired suppression of hepatic glucose output (1). The view that diminished

glucose uptake into fat accounts for decreased whole-body glucose uptake in obesity has recently gained popularity since adipose-selective depletion of the major insulin-responsive glucose transporter, GLUT4, leads to whole-body insulin resistance in transgenic mice (2).

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Abbreviations: AUC, area under the curve; CT, computed tomography; DMEM, Dulbecco's modified Eagle's medium; FFM, fat-free mass; HU, Hounsfield units; KRH, Krebs-Ringer HEPES; LSM, least square means; OGTT, oral glucose tolerance test.

A table elsewhere in this issue shows conventional and Système International (SI) units and conversion factors for many substances.

See accompanying editorial, p. 539.

The notion that adipose tissue plays a key role in glucose homeostasis has also been reinforced by the severe insulin-resistant state found in lipodystrophic individuals (3) and mice (4,5).

Impairment in glucose tolerance is a common feature of human aging (6). Previous studies suggested that this could be due to a defect in insulin secretory capacity and/or insulin action (7,8). In an attempt to elucidate the cellular mechanisms of the insulin resistance associated with aging, Fink et al. (9) and Yki-Jarvinen et al. (10) observed an impairment in insulin-stimulated glucose uptake in isolated adipocytes from middle-aged compared with young subjects. Others have disclaimed that the pathogenesis of age-related glucose intolerance was caused by insulin deficiency and/or resistance (11,12). The transition of young to middle age in humans is commonly characterized by an increase in fat mass (13) and in abdominal adipose tissue accumulation (14,15). It is likely that these body composition changes contribute to the impairment in glucose metabolism occurring during the middle-age period. However, the question of whether age per se influences glucose homeostasis remains controversial. The aims of this study were then 1) to examine the glucose tolerance estimated by an oral glucose tolerance test (OGTT) in young and middle-aged men and women when total or regional adiposity is taken into account and 2) to examine the in vitro glucose uptake in subcutaneous abdominal and visceral adipose tissue explants in a subgroup of young and middle-aged women paired for total and regional adiposity.

RESEARCH DESIGN AND METHODS

Study 1

Subjects. A total of 165 men and 201 women were selected from the Phase II cohort of the Québec Family Study. Caucasian nuclear families from the greater Québec City area were recruited to partic-

ipate in this study, which received approval from the Laval University Medical Ethics Committee. Each participant gave written informed consent before the beginning of the study. All individuals underwent a medical evaluation by a physician, which included a medical history. Subjects with cardiovascular disease, diabetes, endocrine disorders, or those on medication that could have influenced triglyceride metabolism (β -blockers and antihypertensive drugs) were excluded from the analyses. All participants were sedentary (i.e., fewer than two exercise sessions of 30 min/week), nonsmokers, and moderate alcohol consumers (i.e., fewer than 140 g/week). Individuals were classified as "young" when their age was within 18–35 years and as "middle-aged" when they were aged between 50 and 70 years, inclusively.

Anthropometric and computed tomography measurements. Body density was determined by the underwater weighing technique and percent body fat was derived from body density (16). Pulmonary residual volume was measured using the helium dilution method (17). Fat mass was calculated as total body weight minus fat-free mass (FFM). Waist and hip circumferences were measured according to procedures recommended at the Airlie Conference (18). Computed tomography (CT) was performed on a Siemens Somatom DRH scanner (Erlangen, Germany), according to the methodology previously described by Sjöström et al. (19). Briefly, subjects were examined in the supine position with both arms stretched above the head. CT scans were performed at the abdominal (between L4 and L5 vertebrae) level, using an abdominal scout radiograph to establish the precise scanning position.

OGTT. A 75-g OGTT was performed in the morning after an overnight fast. Blood samples were collected in tubes containing EDTA and Trasylol (Miles Pharmaceuticals, Rexdale, ON, Canada) through a venous catheter from an antecubital vein at –15, 0, 15, 30, 45, 60, 90, 120, 150, and 180 min. Plasma insulin concentrations were determined by radioimmunoassay with polyethylene glycol separation (20) (Linco Research, St. Louis, MO), and plasma glucose levels were determined using the glucose oxidase assay (21) (Sigma, St. Louis, MO). The total insulin and glucose areas under the curve (AUCs)

during OGTT were calculated with the trapezoid method.

Study 2

Subjects. Eight young and eight middle-aged women participated in this study, which was reviewed and approved by the institutional ethical committees of the Princess Alexandra Hospital (Brisbane, Australia) and Wesley Hospital (Brisbane, Australia). Informed consent was received from all subjects before participation. Individuals with diabetes, systemic illness, or malignancy or those on medication known to influence glucose metabolism were excluded. Subjects had fasted overnight before adipose tissue removal. Biopsies of adipose tissue were obtained from omental and subcutaneous abdominal sites of patients undergoing elective abdominal surgery at the Wesley Hospital or Princess Alexandra Hospital. Biopsies were obtained at the time of surgery and immediately transported to the laboratory in Dulbecco's modified Eagle's Medium (DMEM) (1,000 mg/l glucose) containing 2% BSA (transport time 30–40 min).

Adipose tissue glucose uptake. As previously described (22), whole tissue adipose explants (15–20 mg), excluding visible connective tissue and blood vessels, were removed from the biopsy material and placed in DMEM (1,000 mg/l glucose) supplemented with 2 mmol/l L-glutamine, 100 units/ml penicillin, 100 μ g/ml streptomycin, and 2% BSA and incubated for 30 min at 37°C under 5% CO₂. Medium was removed and explants were washed three times in Krebs-Ringer HEPES (KRH) buffer containing 1% BSA (pH 7.4) kept at 37°C. Explants were incubated in 0.5 ml KRH buffer with either 0 or 100 nmol/l insulin for 15 min at 37°C under 5% CO₂. Then, 50 μ mol/l 2-deoxyglucose, 0.04 μ mol/l ³H-2-deoxyglucose (0.66 μ Ci/ml), and 42 μ mol/l [¹⁴C]-inulin (0.22 μ Ci/ml) were added to the explants and incubated for a further 20 min at 37°C. We found that a maximally effective stimulation of glucose transport occurred at 20 min using 100 nmol/l insulin (data not shown).

Following incubation, explants were washed in ice-cold KRH buffer to stop transport and washed a further four times in the same buffer in order to remove unbound label. Explants were blotted and then weighed using Mettler scales. ³H and ¹⁴C radioactivity were determined by scintillation counting using a Minaxa

Scintillation counter from Packard. Basal glucose uptake was assessed with 2-deoxyglucose uptake in the absence of insulin. In each experiment, points were determined in triplicate. Data were expressed as picomole 2-deoxyglucose per gram wet weight.

Statistical analyses. The Student's *t* test was utilized for comparisons between young and middle-aged subjects. ANCOVAs were used to determine whether there were significant differences in plasma glucose or insulin between groups once the effect of fat mass or subcutaneous abdominal or visceral adipose tissue area was controlled. The effects of age (young versus middle-aged) and adipose depots (subcutaneous abdominal versus omental) on adipose tissue glucose uptake were tested by a two-way ANOVA for repeated measures. Variables not normally distributed (Shapiro-Wilk test, *P* < 0.05) were transformed mathematically before analyses. All analyses were performed using JMP program (SAS Institute, Cary, NC).

RESULTS

Study 1

Physical characteristics of subjects are presented in Table 1. Young and middle-aged men presented similar body weight, hip circumference, and subcutaneous abdominal tissue accumulation. BMI, waist circumference, fat mass, and visceral adipose tissue were greater in middle-aged than in young men while FFM was greater in the young group (*P* < 0.01). In women, no difference between groups was observed in body weight and hip circumference. However, middle-aged women showed greater BMI, waist circumference, fat mass, and subcutaneous and visceral adipose tissue accumulation and lower FFM than young women (*P* < 0.01).

As shown in Fig. 1, fasting plasma glucose levels were significantly higher in middle-aged as compared with young men (*P* < 0.001). The glucose response following an OGTT significantly increased in both groups; this effect was greater in middle-aged than young men (group \times time interaction, *P* < 0.001). No difference was observed in fasting plasma insulin levels between young and middle-aged men. However, a delayed insulin response to the OGTT was observed in middle-aged as compared with young

Table 1—Physical characteristics of subjects

	Men		Women	
	Young	Middle-aged	Young	Middle-aged
n	84	81	110	91
Age (years)	26 ± 5	58 ± 5†	25 ± 5	57 ± 5†
Body weight (kg)	78 ± 14	79 ± 13	65 ± 16	67 ± 13
BMI (kg/m ²)	25 ± 4	27 ± 4*	25 ± 6	27 ± 6*
Waist (cm)	86 ± 11	95 ± 11†	76 ± 13	84 ± 13†
Hip (cm)	100 ± 7	98 ± 7	100 ± 11	103 ± 11
Fat mass (kg)	15 ± 8	21 ± 7†	18 ± 10	25 ± 9†
FFM (kg)	62 ± 7	59 ± 7*	46 ± 6	42 ± 5*
% Body fat	19 ± 7	26 ± 6†	27 ± 9	36 ± 7†
Abdominal adipose tissue areas (cm ²)				
Subcutaneous	191 ± 126	216 ± 97	267 ± 163	329 ± 134*
Visceral	81 ± 46	155 ± 63†	53 ± 28	121 ± 47†

Data are means ± SD. Significant difference between young and middle-aged subjects at * $P < 0.01$ and † $P < 0.001$.

men (group × time interaction, $P < 0.001$). In women, fasting plasma glucose levels and glucose response to an OGTT were significantly higher in middle-aged women ($P < 0.001$), whereas fasting plasma insulin levels and insulin response were similar between both groups (Fig. 2).

To examine the independent effect of

age on glucose homeostasis, adjustment for fat mass, FFM, and subcutaneous abdominal or visceral adipose tissue accumulation was performed. In both men and women, fasting plasma glucose and glucose response to an OGTT remained significantly higher in middle-aged groups after adjustment for fat mass (least

square means [LSM] of fasting plasma glucose ± SE in young and middle-aged men, respectively: 5.0 ± 0.1 vs. 5.3 ± 0.1 , $P < 0.001$; LSM of glucose response in young and middle-aged men: 1.16 ± 0.03 vs. 1.25 ± 0.03 , $P < 0.05$; LSM of fasting glucose in young and middle-aged women: 4.8 ± 0.1 vs. 5.0 ± 0.1 , $P < 0.01$; LSM of glucose response in young and middle-aged women: 1.07 ± 0.02 vs. 1.20 ± 0.03 , $P < 0.001$). Similar observations were found when plasma glucose and glucose response were corrected for FFM or subcutaneous abdominal tissue area (not shown). As shown in Fig. 3, fasting plasma glucose remained significantly higher in middle-aged as compared with young men after correction for visceral adipose tissue area ($P < 0.01$). On the other hand, no difference between groups was observed in the adjusted glucose response for visceral adipose tissue area. In women, statistical control for visceral adipose tissue accumulation eliminated the original differences found in fasting plasma glucose and glucose response to the OGTT between young and middle-aged women. Similar findings were observed when middle-aged women on hormone replacement therapy ($n = 17$) were excluded from the analyses.

Study 2

Physical characteristics of young and middle-aged women in whom adipose tissue biopsies were performed are as follows: age (years) 31 ± 3 and 57 ± 5 ; body weight (kg) 75 ± 19 and 75 ± 18 ; BMI (kg/m²) 29 ± 8 and 29 ± 8 ; waist (cm) 86 ± 17 and 90 ± 14 ; and hip (cm) 100 ± 11 and 103 ± 11 . The only significant difference observed between groups was age at $P < 0.001$.

Basal and insulin-stimulated 2-deoxyglucose uptake of subcutaneous and omental adipose tissue in young and middle-aged women are presented in Fig. 4. No difference was observed in basal and insulin-mediated glucose uptake between groups. Furthermore, insulin-stimulated 2-deoxyglucose uptake did not significantly differ from basal in both groups and depots, although insulin-stimulated 2-deoxyglucose uptake tended to be greater than basal in subcutaneous abdominal tissue in young women ($P = 0.07$). Finally, no significant depot variation in basal or insulin-stimulated 2-deoxyglucose uptake was observed within each group.

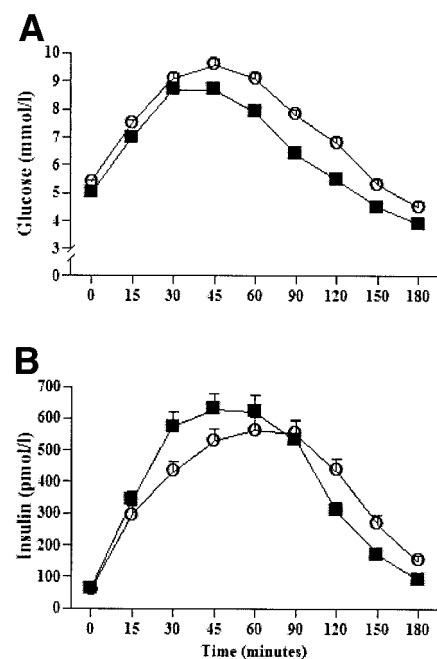


Figure 1—Plasma glucose (A) and insulin (B) levels before and after an OGTT in young (■) and middle-aged (○) men. A: Significant interaction between group and time at $P < 0.001$. B: Significant interaction between group and time at $P < 0.001$. Data are means ± SE.

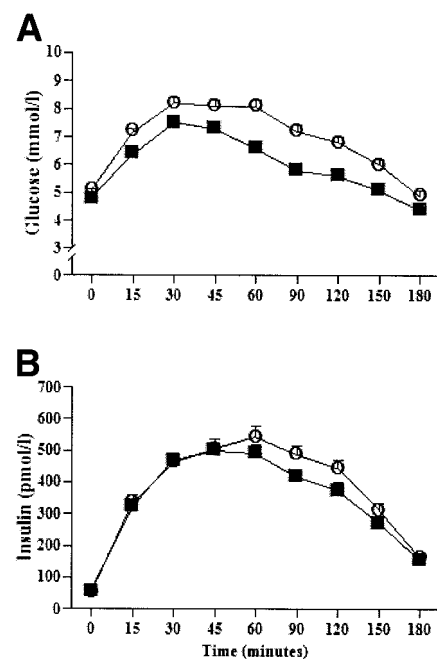


Figure 2—Plasma glucose (A) and insulin (B) levels before and after an OGTT in young (■) and middle-aged (○) women. A: Significant main effect for group ($P < 0.001$) and time ($P < 0.001$). B: Significant main effect for time at $P < 0.001$. Data are means ± SE.

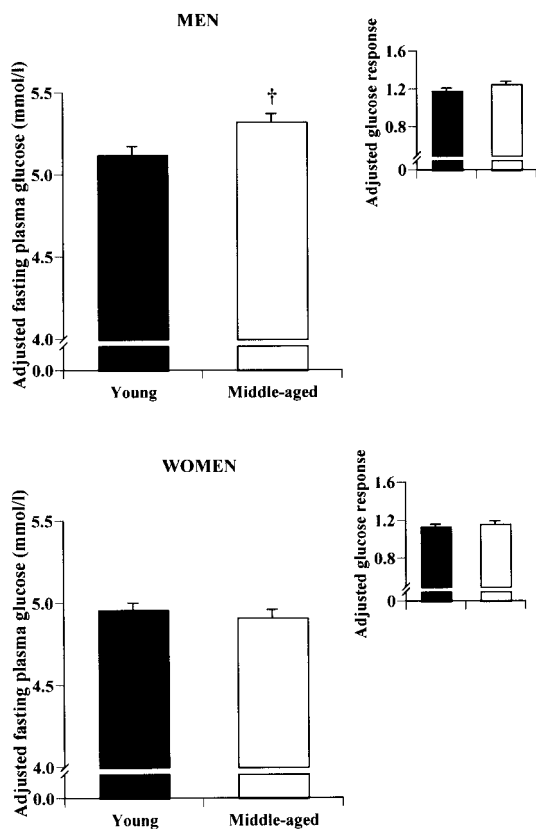


Figure 3— Fasting plasma levels and the response to an OGTT of glucose in young and middle-aged men and women after correction for visceral fat accumulation. Glucose responses are expressed in $\text{mmol/l} \times 10^{-3}$. Data are means \pm SE. Significant difference between groups at $\dagger P < 0.01$.

CONCLUSIONS— This study was undertaken 1) to examine the glucose tolerance estimated by an OGTT in young and middle-aged men and women when total or regional adiposity was taken into account and 2) to examine the in vitro glucose uptake in subcutaneous abdominal and visceral adipose tissue explants in a subgroup of young and middle-aged women paired for total and regional adiposity. We found that fasting plasma glucose and the AUC of glucose following an OGTT were greater in middle-aged as compared with young men and women. These differences in glucose homeostasis between young and middle-aged individuals were not further seen when variables were corrected for visceral adipose tissue accumulation only. In addition, we reported that basal and insulin-stimulated 2-deoxyglucose uptake in subcutaneous abdominal and omental adipose tissue did not significantly differ between young and middle-aged women paired for total and abdominal adiposity.

To our knowledge, this study is one of the largest to estimate glucose tolerance in young and middle-aged men and women in whom regional fat distribution was determined by CT. Consistent with previous

studies (13,14,23), we observed a greater total fat mass and visceral fat accumulation in older men and women. Based on the well-known relationship between obesity and insulin resistance (1), it was not surprising to observe a greater glucose response to the OGTT in middle-aged men and women. The fact that the glucose response to the OGTT did not differ between young and middle-aged subjects only after adjustment for visceral adipose tissue accumulation lends credit to the idea that visceral adipose tissue rather than age per se influences glucose tolerance. The data of the present study are supported by those of a recent study of Ferrannini et al. (24), which showed that the effect of age on insulin action was mainly explained by the age-related changes in body composition. More recently, DeNino et al. (25) also reported that visceral fat contributes in part to the decline in insulin sensitivity measured by a hyperinsulinemic-euglycemic clamp in nonobese women of varying ages. Of note, fasting plasma glucose levels remained higher in middle-aged as compared with young men after correction for visceral fat accumulation in this study. This observation suggests that factors

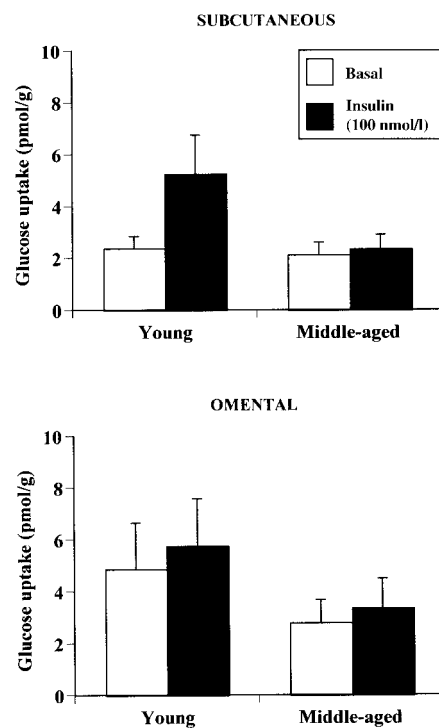


Figure 4— Basal and insulin-stimulated 2-deoxyglucose uptake in subcutaneous abdominal and omental adipose tissue explants in young and middle-aged women paired for total and regional adiposity. Data are means \pm SE.

other than visceral fat accumulation may contribute to the impairment of glucose homeostasis of aging in men. Such factors may be a decreased GLUT4 protein concentration in skeletal muscle (26) and/or a reduced plasma insulin-like growth factor-1 levels (27)—two factors shown to be associated to age-related insulin resistance after anthropometric adjustments.

Theoretically, the altered glucose responsiveness occurring in the middle-age period may be due to altered suppression of glucose production in the liver and/or decreased glucose uptake in muscle and adipose tissues. Previous reports have shown that aging was associated with a significant defect in glucose uptake in adipocytes (9,10). The contribution of body fat accumulation and/or distribution were nevertheless not taken into account in these studies. This notion appears important since abdominal, gluteal, and omental adipocytes were found to be less responsive to the stimulatory effect of insulin on glucose uptake in upper-body as compared with lower-body obese individuals (22,28). To our knowledge, the present study is the first to examine the

effect of age per se on insulin-mediated glucose uptake in subcutaneous and omental adipose tissue explants of young and middle-aged women paired for body fat accumulation and distribution. At least in women, no significant difference in basal and insulin-stimulated glucose uptake was noted between young and middle-aged. This observation suggests that age per se does not alter basal and insulin-mediated glucose uptake in subcutaneous and omental adipose tissue in women. Because adipose tissue could only be collected from women, further studies need to be performed in men.

In conclusion, these results suggest that 1) visceral obesity rather than age per se seems to be responsible for the commonly observed decrease in glucose tolerance in middle-aged subjects, and 2) aging does not appear to be associated with defects in *in vitro* glucose uptake in subcutaneous abdominal and omental adipose tissue in young and middle-aged women paired for total and abdominal adiposity.

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