Sequential hyperglycemic-euglycemic clamp to assess β-cell and peripheral tissue: studies in female athletes

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Received 29 September 2000; accepted in final form 3 April 2001

Ryan, Alice S., Denis C. Muller, and Dariush Elahi. Sequential hyperglycemic-euglycemic clamp to assess β-cell and peripheral tissue: studies in female athletes. J Appl Physiol 91: 872–881, 2001.—Insulin secretion and rate of utilization (Rd) of glucose were tested during a newly developed sequential clamp in 42 highly trained female athletes (A; 18–69 yr old) and 14 sedentary control women (C; 18–50 yr old; body mass index <25 kg/m2). The A women were categorized into four age groups: 18–29, 30–39, 40–49, and 50–69 yr old. The C women were also grouped by age (18–29 and 40–50 yr old). During the three-step clamp (hyperglycemia, return to euglycemia, and hyperinsulinemia), glucose turnover was assessed with [3-3H]glucose. Among the A, the youngest group had the largest first- and second-phase insulin response, which was significantly different from the oldest A (P < 0.05). Among the two C groups, first-phase response of both groups and second-phase response of the older group was higher than respective age-matched A (P < 0.05). During the hyperglycemic period, glucose Rd was similar among A groups and between A and C. Despite similar levels of insulin between groups during the hyperinsulminemic period (~400 pmol/l), A utilized 36% more glucose than C (P < 0.001). Glucose Rg was not different across the age groups of A. This newly developed sequential clamp procedure allows assessment of both β-cell sensitivity to glucose and peripheral tissue sensitivity to insulin in a single session. We have shown that physical activity improves β-cell efficiency across the age span in women and ameliorates the effect of age on the decline of peripheral tissue sensitivity to insulin.

NORMAL AGING IS CHARACTERIZED by a progressive impairment in carbohydrate intolerance (3) and usually by a reduction in physical activity (11). A sedentary lifestyle, prevalent in aging, is associated with increased obesity and a loss of fat-free mass (FFM), conditions that promote insulin resistance and hyperinsulinemia. Our laboratory previously reported that intense chronic exercise in female athletes prevented both the decline in FFM with age and the increase in body fat (28). Physically trained men have higher insulin sensitivity and glucose effectiveness and uptake than sedentary controls (15, 26, 35). Moreover, endurance training reduces hyperinsulinemia and improves insulin action in older sedentary men and women (17, 36). Thus far, no studies are published that have examined the effects of high physical fitness on glucose homeostasis in women. Furthermore, the influence of aging on glucose utilization in physically trained women is unknown. The purpose of the present study was to determine β-cell sensitivity to glucose and peripheral tissue sensitivity to exogenous insulin in highly trained female athletes across the age span in a single session. We hypothesized that, in female athletes, the deterioration of glucose intolerance that is associated with aging is prevented. A glucose clamp, consisting of a hyperglycemic clamp followed by recovery to basal glucose level and then immediately succeeded by a hyperinsulminemic-euglycemic clamp, was specifically developed for this investigation to test these parameters in this group of women. This study shows, for the first time, that peripheral tissue sensitivity is preserved in older female athletes.

METHODS

Subjects

Sixty-seven women (53 athletes and 14 controls) between the ages of 18–69 yr with body mass index (BMI) <25 kg/m2 were recruited for participation in the study. Athletes were swimmers, runners, and triathletes who were training for collegiate, local, and national competitions. Athletes trained on average 5–6 days/wk for 12 h/wk. The youngest swimmers averaged 60,000 yards/wk at intensities up to 1 min/100 yards. Other athletes who swam averaged 14,000 yards/wk at an intensity of ~1 min and 20 s/100 yards. All runners averaged 27–30 miles/wk at an average 7–8 min/mile pace. Some of these athletes also cycled at 18 miles/h for ~65–95 miles/wk. The 14 control volunteers were healthy sedentary women who had not participated in a regular exercise program for a minimum of 6 mo before the study. All women were weight stable (no weight change of >2 kg for the previous 2 wk). Subjects were screened by medical history, physical examination, fasting blood profile, 2-h oral glucose tolerance test, and a graded exercise treadmill test. All sub-

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jects were nonsmokers, free of diabetes (7) and cardiovascular disease (by history and physical examination as well as treadmill stress test), and not on any medications known to influence glucose metabolism. In addition, to validate the use of a sequential hyperglycemic clamp, return to euglycemia, followed by hypoglycemia, we performed hyperinsulinemic-euglycemic clamps, without the preceding hyperglycemic clamp, in two groups of volunteers and compared the rate of disappearance (Ra) of glucose. The first group consisted of 10 sedentary volunteers who were matched with respect to age and BMI to the young control group. The second group consisted of four Type 2 diabetic volunteers. The values of glucose Ra of this latter group were compared with those from additional diabetic volunteers, again matched for age and BMI, who were studied with the sequence hyperglycemic, return to basal, and hyperinsulinemic clamps. The volunteers who were studied for the purpose of validation of the sequential clamps were not examined with respect to body composition or maximal oxygen consumption (V\textsubscript{O2 max}). All methods and procedures were approved by the Institutional Review Board of the University of Maryland. All subjects provided written informed consent.

**Body Composition**

Fat mass, lean tissue mass, and bone mineral content were determined by dual-energy X-ray absorptiometry (model DPX-L, LUNAR Radiation, Madison, WI). FFM was calculated as lean tissue plus bone mineral content. All dual-energy X-ray absorptiometry scans were analyzed using the LUNAR Version 1.3z DPX-L program for body composition analyses. To quantify visceral and abdominal subcutaneous fat, computerized tomography scanning of the abdomen was performed using a General Electric High Speed Advantage 9800 Scanner as previously described (28).

**V\textsubscript{O2 max}**

V\textsubscript{O2 max} was measured during a progressive treadmill test to subjective exhaustion as previously described (29). Validation for attainment of V\textsubscript{O2 max} included meeting two of the following three criteria: 1) a plateau in oxygen uptake with an increased workload as evidenced by a difference in oxygen uptake of $\geq 2$ ml·kg$^{-1}$·min$^{-1}$, 2) a respiratory exchange ratio $> 1.10$, and 3) a maximal heart rate within 10 beats/min of the age-predicted maximal value. Athletes were categorized into four age groups: 18–29, 30–39, 40–49, and 50–69 yr old. To qualify for the study, athletes had to reach a V\textsubscript{O2 max} of $\geq 50, 45,$ and $40$ ml·kg$^{-1}$·min$^{-1}$ for ages $\leq 39, \leq 49,$ and $\geq 50$ yr, respectively. Swimmers were allowed a 5 ml·kg$^{-1}$·min$^{-1}$ difference in these criteria because V\textsubscript{O2 max} is underestimated when they are not tested swimming (1). Forty-two of the athletes met the criteria for aerobic capacity and were enrolled into the study. The 14 control women were also grouped by age (18–29 and 40–50 yr). All controls had to have a V\textsubscript{O2 max} $< 40$ ml·kg$^{-1}$·min$^{-1}$.

**Hyperglycemic/ Hyperinsulinemic-Euglycemic Clamps**

All subjects were weight and activity stabilized before metabolic testing. A newly developed three-step clamp was performed in all volunteers. In athletes, all clamps were performed 24–36 h after the last exercise bout. Subjects consumed at least 200 g carbohydrate for 3 days before testing. A triple-lumen polyethylene catheter was inserted by percutaneous venipuncture for infusion of trigitated glucose, unlabeled glucose, and insulin. A second catheter was inserted in a retrograde fashion into a dorsal hand or wrist vein, and the hand was enclosed in a grounded, insulated chamber, warmed to 70°C to “arterialize” (20) the blood obtained for all samples. Two hours before the start of the clamp (−120 min), a priming dose of [3-\textsuperscript{3}H]glucose (8.37 kBq/kg) was administered, followed by continuous infusion of [3-\textsuperscript{3}H]glucose (87.32 Bq·kg$^{-1}$·min$^{-1}$) for the duration of the experiment (to 270 min). Steady-state glucose specific activity was achieved by 90 min. To confirm this and to assess basal glucose and insulin levels, four arterialized blood samples were obtained at 10-min intervals starting at −30 min. With the start of the clamp at time 0, blood samples were obtained every 2 min from 0 to 10 min and every 5 and 10 min thereafter for the determination of plasma glucose and insulin levels. The clamp consisted of three steps: 1) a hyperglycemic clamp for 1 h (+5.4 mmol/l above basal) followed by 2) 1 h of glycemic recovery to basal glucose level and immediately followed by 3) a hyperinsulinemic-euglycemic clamp for 2 h followed by 0.5 h of recovery. The 10-min priming and continuous infusion of insulin (240 pmol·m$^{-2}$·min$^{-1}$; Humulin, Eli Lilly, Indianapolis, IN) have been previously described (6). This resulted in a square wave of insulinemia at a level of $\approx 400$ pmol/l among all groups. At 240 min the insulin infusion was stopped, but plasma glucose levels were maintained at basal levels for the next 30 min. During the hyperinsulinemic-euglycemic portion of the clamp (step 3), the 20% glucose solution was spiked with tritiated glucose, “hot Ginf,” to maintain a stable plasma glucose specific activity (12). The plasma glucose levels were well maintained and stable during each clamp period and in all studies (n = 56) averaged 101.1 ± 1.20 and 99.3 ± 1.50% (SD) of the desired goal for steps 1 and 3, respectively. The coefficient of variation of plasma glucose levels in the 56 clamps was 3.3 ± 1.3 and 5.6 ± 1.8 (SD) for steps 1 and 3, respectively. The actual concentration of the glucose solution measured 10.20 ± 0.03 mmol/l, which was 90% of its stated concentration. For the validation study, insulin was infused from 0 to 120 min. The dose of insulin used in the diabetic volunteers was 480 pmol·m$^{-2}$·min$^{-1}$ because of the well-established insulin resistance associated with this condition. This dose was also used in the sequential hyperglycemic, return to basal, and hyperinsulinemic clamp. In the latter clamp, the protocol was identical to those used in the athletes except for the insulin dose. In all diabetic volunteers, during the insulin infusion period (0–120 or 120–240 min), plasma glucose was allowed to fall to 5.5 mmol/l before the start of the glucose infusion (hot Ginf) and the plasma glucose level was maintained stable at 5.3 mmol/l thereafter. In all validation studies, the coefficient of variation of plasma glucose, during the hyperglycemic part (in the diabetic volunteers) or the euglycemic portion part of the study (in both sedentary and diabetic volunteers) did not exceed 5% in any volunteer.

**Indirect calorimetry.** To quantitate carbohydrate oxidation, continuous indirect calorimetry was performed before the start of the glucose infusion and during the last 30 min of the insulin infusion by the open-circuit dilution technique using a SensorMedics DeltaTrac cart (Yorba Linda, CA). Rates of glucose oxidation were calculated from measurements of carbon dioxide production and oxygen consumption using established equations (19) with correction for protein oxidation determined from 24- or 12-h (corrected for 24 h) urinary urea nitrogen. Nonoxidative glucose metabolism was calculated as the difference between total glucose uptake and glucose oxidation.

**Analysis of blood samples.** Blood samples were collected in heparinized syringes. Plasma glucose was measured with the glucose oxidase method (Beckman Instruments, Fullerton, CA). Immuno reactive insulin was determined by an insulin-
specific double-antibody system as previously described (30) using human insulin standards and tracer. The antisera was raised against highly purified human insulin and does not cross-react with human proinsulin (<0.1%) (Linco, St. Louis, MO). The lower limit of detection of this assay is 12 pmol/l. Intra- and inter assay coefficients of variation of pooled control sera average 5 and 9%, respectively. Specific activity of [3-3H]glucose was determined on deproteinized and evaporated aliquots of plasma using the Somogyi-Nelson method of deproteinization (33).

Statistical Analyses

The rates of total appearance (Ra) and Rd of glucose were calculated according to the non-steady-state equations of Steele (34) as modified for the use of hot Ginf (12). The volume of distribution of glucose was assumed to be 210 ml/kg (14). During the basal period, glucose Ra is equal to hepatic glucose production because the liver is the principal source of glucose. When glucose is infused (i.e., during the clamp), endogenous glucose production is estimated as the difference between its calculated total glucose Ra and exogenous glucose infusion rate for the appropriate time interval.

All data were analyzed using the Statistical Analysis System version 6.07 (31). Standard methods were used to compute means, standard errors of the mean, and Pearson correlation coefficients. The mean concentration of glucose and insulin was calculated for each sample time point. The trapezoidal rule was used to calculate the integrated response of the first-phase insulin release (0–10 min) and over 30-min intervals for each subject. The integrated response was divided by its time interval (10 or 30 min) to compute mean concentrations. Differences between groups were evaluated using unpaired t-tests and repeated-measures analysis of variance. Analysis of covariance was employed to adjust for differences in age, obesity, and fat distribution when differences between groups were analyzed. Multiple regression was used to examine the relationship of glucose uptake and insulin response. All statistical tests were two-tailed. Except where otherwise stated, data are expressed as means ± SE, and P values below 0.05 were regarded as statistically significant.

RESULTS

Subject Characteristics

The 42 female athletes were of a wide age range (18–69 yr) but had similar BMI values of −21 kg/m² across the age span. A complete description of the body composition characteristics has been previously reported (28). Briefly, weight, FF, and percent body fat were comparable among the groups of athletes, whereas the women in the control group had significantly higher weight, percent body fat, and total fat mass and lower FF than the athletes (all P < 0.05; Table 1). Intra-abdominal fat (IAF) and subcutaneous abdominal adipose tissue were significantly higher in controls than athletes (both P < 0.005) (28). In addition, VO₂ max (ml·kg⁻¹·min⁻¹) was significantly higher in athletes than controls (P < 0.0001).

Glucose Clamps

Figure 1 is a graphic representation of the glucose and insulin responses as well as the glucose infusion during the 4.5-h clamp. The group depicted is the 30- to 39-yr-old athletes. The basal plasma glucose level (5.2 ± 0.88 mmol/l) was rapidly raised by 5.4 mmol/l, and a square wave of hyperglycemia was established from 0 to 60 min (10.9 ± 0.08 mmol/l; Fig 1A). Basal insulin levels averaged 33 ± 1.9 pmol/l (Fig 1B). In response to the square wave of hyperglycemia, a bi-modal release of insulin occurred in each subject. The peak insulin response during the first phase (0–10 min) occurred at min averaging 101 ± 13.4 pmol/l. The second-phase insulin response increased during the first hour, reaching a level of 91 ± 12.9 pmol/l at 60 min. Coincident with the termination of the glucose infusion at 60 min, both plasma glucose and insulin levels began to fall. At 120 min, a square wave of hyperinsulinemia was then created for 2 h. After the termination of the insulin infusion at 240 min, insulin levels declined toward basal from 240–270 min. The corresponding glucose infusion required to maintain hyperglycemia and euglycemia during this variant of the clamp procedure is shown in Fig. 1C. In a few volunteers (athletes), the glucose infusion was started before 120 min to prevent the plasma glucose from falling below the basal state.

Plasma glucose levels were maintained stable at the desired goal for each subject during hyperglycemic and euglycemic steps of the clamp. The mean plasma glucose level during 10–60 min of the hyperglycemic clamp and 120–240 min of the euglycemic clamp was computed for each individual study and expressed as a percentage of the desired goal. The mean glucose level

<table>
<thead>
<tr>
<th>Table 1. Subject characteristics</th>
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<tr>
<td>Athletes</td>
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<tr>
<td>18–29 yr old</td>
</tr>
<tr>
<td>(n = 13)</td>
</tr>
<tr>
<td>Age, yr</td>
</tr>
<tr>
<td>Body weight, kg</td>
</tr>
<tr>
<td>Body fat, %</td>
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<tr>
<td>Fat-free mass, kg</td>
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<tr>
<td>VO₂ max, ml·kg⁻¹·min⁻¹</td>
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Values are means ± SE; n: no. of subjects. A, athlete; C, control; VO₂ max, maximal oxygen consumption. %Body fat was determined by dual-energy X-ray absorptiometry. All at least P < 0.05: * 40- to 50-yr-old C vs. 40- to 49-yr-old A; † 40- to 50-yr-old C vs. 18- to 29-yr-old C; ‡ 30- to 39-yr-old A vs. 18- to 29-yr-old A; § 18- to 29-yr-old C vs. 18- to 29-yr-old A; † 50- to 69-yr-old A vs. 18- to 29-yr-old A. A 55-yr-old marathon runner had a value of 37.
was 10.77 ± 0.06 (SD) and 5.17 ± 0.05 mmol/l in all athletes for the hyperglycemic and euglycemic periods, respectively. For the controls, these levels were 10.70 ± 0.09 and 5.08 ± 0.09 mmol/l during these two periods. There were no differences in glucose levels between the four groups of athletes or the control groups (data not shown).

Fasting plasma glucose and insulin concentrations were similar among the athlete groups and between the athletes and the controls. First-phase insulin response (0–10 min) was not different between the 18- to 29-, 30- to 39-, and 40- to 49-yr-old athlete groups (Table 2). However, first-phase insulin response was significantly lower in the oldest athletes (50–69 yr old) than all other athlete groups (P < 0.05). In addition, the insulin response during the first phase was significantly higher in 18- to 29- and 40- to 50-yr-old controls than the 18- to 29- and 40- to 49-yr-old athletes (both P < 0.05). The second-phase insulin response (10–60 min) was greater in the 18- to 29-yr-old athletes than the other athlete groups (P < 0.001; Fig. 2). There were no significant differences in the second-phase insulin response between the 30-to 39-, 40- to 49-, and 50- to 69-yr-old athletes. The second-phase insulin response (10–60 min) was significantly higher in the 40- to 50-old yr controls than 40- to 49-yr-old athletes (P < 0.01) but was not different between the 18- to 29-yr-old controls and athletes (Fig. 3).

Basal glucose Ra was not different among the athletes or in the control groups (Table 2). During the hyperglycemic period, the recovery period and the hyperinsulinemic-euglycemic period of the clamp, glucose Ra was totally suppressed in all groups (data not shown).

Glucose Rd, which increased over the 1 h of hyperglycemia, was similar among and between athletes and controls up to age 50 yr (Table 2). However, the oldest athletes (50–69 yr) had a significantly lower glucose uptake than the youngest athletes. The glucose Rd values between 90 and 120 min of the recovery period

### Table 2. Insulin, glucose Ra, and glucose Rd responses during the clamps

<table>
<thead>
<tr>
<th></th>
<th>Athletes</th>
<th>Controls</th>
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<tbody>
<tr>
<td></td>
<td>18–29 yr old (n = 13)</td>
<td>30–39 yr old (n = 9)</td>
</tr>
<tr>
<td>Plasma insulin, pmol/l</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Basal</td>
<td>44 ± 3</td>
<td>33 ± 2</td>
</tr>
<tr>
<td>0–10 min</td>
<td>82 ± 7</td>
<td>70 ± 6</td>
</tr>
<tr>
<td>10–60 min</td>
<td>156 ± 15</td>
<td>79 ± 10</td>
</tr>
<tr>
<td>120 min</td>
<td>76 ± 9</td>
<td>58 ± 7</td>
</tr>
<tr>
<td>180–240 min</td>
<td>350 ± 17</td>
<td>384 ± 34</td>
</tr>
<tr>
<td>Basal glucose Ra, μmol·kg⁻¹·min⁻¹</td>
<td>13.54 ± 0.73</td>
<td>13.76 ± 0.58</td>
</tr>
<tr>
<td>Rd, μmol·kg⁻¹·min⁻¹</td>
<td></td>
<td></td>
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<tr>
<td>FFM kg⁻¹·min⁻¹</td>
<td></td>
<td></td>
</tr>
<tr>
<td>30–60 min</td>
<td>41.55 ± 2.17</td>
<td>31.61 ± 1.92</td>
</tr>
<tr>
<td>90–120 min</td>
<td>22.52 ± 1.55</td>
<td>21.78 ± 1.25</td>
</tr>
<tr>
<td>180–240 min</td>
<td>70.56 ± 4.47</td>
<td>79.17 ± 5.16</td>
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</table>

Values are means ± SE; n, no. of subjects. Ra, rate of appearance; Rd, rate of disappearance; FFM, fat-free mass; All at least P > 0.05.

*a* 50- to 69-yr-old A vs. 18- to 29-, 30- to 39-, and 40- to 49-yr-old A; *b* 18- to 29-yr-old C vs. 18- to 29-yr-old A; *c* 40- to 50-yr-old C vs. 40- to 49-yr-old A; *d* 50- to 69-yr-old A vs. 18- to 29-yr-old A; *e* 18- to 29-yr-old A vs. 30- to 39-, 40- to 49-, and 50- to 69-yr-old A.
were similar among the groups of athletes and between athletes and controls.

During the third step of the clamp, plasma insulin levels averaged 396 ± 12 pmol/l among all groups (n = 56) (Table 2). The magnitude of the increase in plasma insulin achieved during the insulin infusion was not significantly different between groups. Rates of overall glucose disposal increased progressively during the first hour hyperinsulinemic-euglycemic period in all groups and was relatively stable during the last hour (180–240 min) of the clamp, averaging 76.87 ± 2.20μmol·kg FFM⁻¹·min⁻¹ in all athletes and 65.76 ± 4.32μmol·kg FFM⁻¹·min⁻¹ in all controls. Glucose Rd during this period was significantly lower in the older controls than the older athletes (40- to 50-yr-old groups; *P < 0.005; Fig. 4) but was not significantly different between young athletes and controls (18- to 29-yr-old groups). In addition, glucose Rd values during this last hour of the hyperinsulinemic-euglycemic clamps were not different across the age groups of athletes (Fig. 5). In both athletes and controls in all age groups, despite the precipitous fall in plasma insulin levels during the recovery period (240–270 min), glucose Rd remained elevated and was not different from the previous 30-min period (210–240 min).

Basal glucose oxidation was similar among athletes and controls and averaged 10μmol·kg FFM⁻¹·min⁻¹ (Table 3). In response to the insulin infusion, glucose oxidation increased in all groups and represented ~36.8 ± 1.2% of glucose disposal during the last 30

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**Fig. 2.** Plasma insulin response during the hyperglycemic and recovery portions of the 3-step clamp (0–120 min) in female athletes. Values are means ± SE. See METHODS for details. *P < 0.001, 18- to 29-yr-old athletes vs. 30- to 39-, 40- to 49-, and 50- to 69-yr-old athletes.

**Fig. 3.** Second-phase (10–60 min) insulin response to hyperglycemia (~11 mmol/l) in the athlete and control groups. Values are means ± SE. *P < 0.001, 18- to 29-yr-old athletes vs. 30- to 39-, 40- to 49-, 50- to 69-yr-old athletes. †P < 0.01, 40- to 50-yr-old controls vs. 40- to 49-yr-old athletes.

**Fig. 4.** Rate of utilization (Rd) of glucose during the 3-step clamp in 18- to 29-yr-old (A) and 40- to 50-yr-old (B) athletes and controls. Values are means ± SE. See METHODS for details. *P < 0.005, 40- to 49-yr-old athletes vs. 40- to 50-yr-old controls.
min of the insulin infusion. Nonoxidative glucose metabolism also increased during this period, and in general glucose storage was greater in the athletes than controls. Young controls (18–29 yr old) oxidized more glucose than the young athletes (18–29 yr old), and the older controls (40–50 yr old) stored less glucose than the 40- to 49-yr-old athletes.

First- and second-phase insulin response correlated with obesity and fitness. In univariate analyses for the combined groups, total fat mass, percent body fat, and subcutaneous abdominal fat explained 13% of the variance in first-phase insulin response ($r = 0.36, r = 0.38, r = 0.34, P < 0.01, P < 0.01$, and $P < 0.05$, respectively). However, first-phase insulin response was not associated with body weight, FFM, or IAF ($n = 54$). The 0- to 10-min insulin response was negatively correlated with $V\dot{O}_2\text{max}$ ($r = -0.37, P < 0.01$; Fig. 6A). The second-phase insulin response correlated positively with fat mass and percent fat and negatively with $V\dot{O}_2\text{max}$ ($r = 0.33, r = 0.34, r = -0.28$; all $P < 0.05$).

Because plasma insulin and glucose levels were similar for each individual during the 180- to 240-min period of the study, the glucose utilization represents a relative index of peripheral tissue sensitivity to insulin. A two-way ANOVA was performed to examine the effect of age, activity level and their interaction on the 180- to 240-min glucose $R_d$. Only the 18- to 29- and 40- to 49-yr-old groups of athletes and the corresponding age-grouped controls were included in the analysis. A statistically significant interaction between age and activity was found ($P < 0.05$). This finding and the fact that the glucose $R_d$ was significantly lower in the older controls suggest that activity increases peripheral tissue sensitivity to insulin and also is protective in maintaining tissue sensitivity with age.

To examine the overall effect of body composition and fitness on $\beta$-cell sensitivity to glucose (both first-

Table 3. Glucose oxidation and storage during the clamps

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<tr>
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<th>Athletes</th>
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<tbody>
<tr>
<td></td>
<td>18–29 yr old</td>
<td>30–39 yr old</td>
</tr>
<tr>
<td></td>
<td>$(n = 12)$</td>
<td>$(n = 9)$</td>
</tr>
<tr>
<td>Glucose oxidation</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Baseline</td>
<td>8.22 ± 1.3</td>
<td>10.3 ± 1.1</td>
</tr>
<tr>
<td>210–240 min</td>
<td>26.7 ± 2.1</td>
<td>27.3 ± 2.0</td>
</tr>
<tr>
<td>Glucose storage</td>
<td>(nonoxidative)</td>
<td></td>
</tr>
<tr>
<td>210–240 min</td>
<td>44.9 ± 3.9</td>
<td>55.2 ± 4.2</td>
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</table>

Values are means ± SE in $\mu$mol·kg FFM$^{-1}$·min$^{-1}$; $n$, no. of subjects. All at least $P < 0.05$: *18- to 29-yr-old C vs. 18- to 29-yr-old A; †50- to 69-yr-old A vs. 18- to 29-yr-old A; ‡40- to 50-yr-old C vs. 40- to 49-yr-old A.
and second-phase responses) and peripheral tissue sensitivity to insulin (glucose $R_\text{d:} 180-240$ min), multiple-regression analyses were performed where the dependent variables entered into the model were BMI, weight, $V_{\text{O}_2\text{max}}$, IAF, subcutaneous abdominal fat, and percent fat for all athletes and control volunteers. Using the backward-elimination procedure, only percent fat remained in the model for first-phase insulin release ($P < 0.008$) and both IAF ($P < 0.44$) and percent fat ($P < 0.002$) remained in the model for second-phase insulin release. For the peripheral tissue sensitivity analysis ($R_\text{d:} \mu\text{mol-kg}^{-1}\cdot\text{min}^{-1}$), age was included in the model to adjust for its effect between the two groups. Only $V_{\text{O}_2\text{max}}$ ($P < 0.0001$) remained in the model in addition to the age effect ($P < 0.04$). This relationship did not change when $R_\text{d}$ was expressed per FFM instead of total kilogram of body weight.

Glucose uptake during the last hour of the hyperinsulenic-euglycemic period in the total group ($n = 56$) was positively associated with FFM ($r = 0.34$, $P < 0.05$), negatively associated with total fat mass ($r = -0.52$, $P < 0.001$), IAF ($r = -0.36$, $P < 0.01$), and subcutaneous abdominal fat ($r = -0.50$, $P < 0.0005$), but not associated with body weight. $V_{\text{O}_2\text{max}}$ was strongly associated with $R_\text{d}$ from 180 to 240 min ($r=0.53$, $P < 0.0005$, Fig. 6B).

Validation Studies

Rates of overall glucose disposal in the 10 volunteers, who were similar in age and BMI to the 8 volunteers of the young sedentary group, assessed with the hyperinsulenic-euglycemic clamp without the preceding hyperglycemic clamp averaged $42 \pm 6 \mu\text{mol-kg}^{-1}\cdot\text{min}^{-1}$ during the 90- to 120-min period. This was not different from the glucose $R_\text{d}$ of the eight volunteers during the 180- to 240-min period assessed with the sequential clamp. Glucose $R_\text{d}$ assessed with the hyperinsulenic-euglycemic clamp in four diabetic volunteers averaged $28 \pm 2.4 \mu\text{mol-kg}^{-1}\cdot\text{min}^{-1}$ during the 90- to 120-min period. Glucose $R_\text{d}$ of the four diabetic volunteers, with a similar age and BMI as the above four diabetic volunteers, averaged $23 \pm 4.6 \mu\text{mol-kg}^{-1}\cdot\text{min}^{-1}$ during the 210- to 240-min period as assessed with the sequential clamp.

DISCUSSION

The present investigation determined, for the first time, that $\beta$-cell sensitivity to glucose and peripheral tissue sensitivity to insulin is preserved among athletes as a function of age. In general, after examination of both phases of insulin release, we conclude that in highly trained female athletes, $\beta$-cell sensitivity is maintained across the age span. Glucose uptake, determined with the euglycemic clamp technique, was notably similar between young and older athletes despite the large difference in age.

Our results confirm observations that endurance-trained subjects have a lower plasma insulin response to a given glucose stimulus than untrained individuals (15, 32) and that those who are aerobically trained have increased rates of glucose disposal (15, 23, 24). Our new technique confirms these findings in a unique group of highly trained female athletes across the adult age span. In the present study, the older sedentary women had a 70% greater first-phase and 103% greater second-phase insulin response during hyperglycemia than the athletes. In addition, despite the equivalency of hyperinsulinemia during the last hour of the euglycemic period, the older athletes utilized on average 31% more glucose than similarly aged sedentary women, suggesting an increase in insulin sensitivity due to the effects of training.

In general, the results from the hyperglycemic clamp of this study indicate that first-phase insulin response declines with age in female athletes, whereas the late-phase insulin release does not after age 30 yr. In the youngest athletes, the second-phase insulin response is the highest with no differences observed between the other age groups of athlete. In contrast, among healthy men and women aged 21–74 yr, DeFronzo et al. (4) found no difference in either the early or late insulin secretion during hyperglycemic clamps between young and old adults. Our laboratory has also previously reported no differences in secretory response in persons aged >25 yr (5). Although the mean age of our youngest group is 20 yr and slightly lower than the above two studies, we cannot explain the disparate findings. Of note, the young controls with a similar mean age also had a high first-phase insulin response.

The increase in obesity (10) and central body fat (9) associated with aging may be altered by increased levels of physical activity. Furthermore, obesity, in particular abdominal obesity, is associated with reduced rates of glucose uptake (18). Our laboratory has previously shown that these highly trained older female athletes can prevent a decline in FFM but not an increase in IAF fat (28). In the present investigation, FFM and IAF were not associated with either first- or second-phase insulin secretion. Total body fat was, however, associated with the insulin response to hyperglycemia. Thus the total amount of fat may be more important than visceral fat in the insulin release during hyperglycemia even in very lean women. The insulin release in response to hyperglycemia was also negatively associated with physical fitness.

The usual decline in peripheral tissue sensitivity to insulin normally observed with aging (21) may have been prevented in the older female athletes by the high levels of training, reduced body fat, and maintenance of muscle mass. Glucose uptake is directly proportional to muscle tissue and inversely proportional to percent body fat in young male runners (38). In the present investigation, glucose uptake was positively associated with FFM and negatively related to low levels of total and abdominal body fat. In addition, we confirm that aerobic capacity is associated with greater peripheral tissue sensitivity to insulin (15). Thus both physical fitness and muscle mass are related to glucose utilization and probably contribute to the enhanced tissue sensitivity observed in these female athletes.
The observation that, at the dose employed, there was no difference in the magnitude of the increase in plasma insulin during the 2-h hyperinsulinemic-euglycemic period among and between groups indicates that, at this concentration, the insulin clearance rate was similar across the age span in women. Other studies have shown a difference in the insulin clearance rate as a function of age (8, 27). However, these studies were performed in untrained men and over much higher concentrations of insulin.

Not only does the amount of muscle mass contribute to the high glucose utilization for these female athletes but also specific changes in muscle characteristics and its properties likely play a role. A possible mechanism is an increase in the level of GLUT-4 transporters in skeletal muscle, the major isoform responsible for insulin-stimulated glucose uptake. We have previously shown that endurance training increases GLUT-4 in older impaired glucose-intolerant men and women (13). Other potential mechanisms include alterations in key enzymatic processes that regulate glucose uptake and storage as well as interactions between glucose and esterified free fatty acid metabolism, notably enhancement of glucose transport, phosphorylation and glycogen synthesis via GLUT-4, hexokinase II, glucokinase, and glycogen synthase mechanisms (2).

A possible confounder in the differences observed between athletes and controls is the effect of the last bout of exercise. However, because all athletes were tested under the same conditions (i.e., the clamps were performed 24 h after the last exercise bout), this should not have influenced our conclusions with respect to aging and glucose metabolism in the athletes alone. The athletes in our study exercised approximately every day of the week so that studies performed 24 h after a training session represents their typical lifestyle. The effects of an acute bout of exercise on insulin secretion and glucose uptake in untrained and trained individuals has been examined (16, 22, 23, 35). An acute bout of treadmill exercise in untrained men increased early-phase insulin secretion but did not affect insulin levels during the late phase of a hyperglycemic clamp (16). Others report no change in the insulin secretion in untrained subjects after moderate exercise (22) but increased insulin-mediated glucose uptake immediately and 48 h after exercise in untrained subjects (23). In contrast, glucose uptake was not enhanced after an acute bout of exercise in trained subjects (24). Furthermore, young trained runners had a higher glucose effectiveness and insulin sensitivity than sedentary subjects both 16 h and after refraining from training for 1 wk (35), suggesting that long-term adaptations to training occurred in the runners. These studies imply that an acute bout of exercise may affect glucose metabolism more in sedentary individuals than those who are already active.

The design of the present study allowed us to examine both β-cell sensitivity to glucose and peripheral tissue sensitivity to exogenous insulin in each individual during a single study. This combination of a hyperglycemic clamp and hyperinsulinemic-euglycemic clamp has the advantage of being able to examine the many facets of glucose homeostasis in one test. It is plausible that the rate of glucose utilized during the hyperinsulinemic-euglycemic step of the clamp was influenced by stimulation of the β-cell by glucose during the first step of the clamp. This could occur through alterations in glycogen stores, NADPH/NADP, NADH/NAD, and other regulatory intermediates (e.g., hormones). Thus glucose R\textsubscript{b} during the second step may not be comparable to glucose R\textsubscript{b} obtained when only a hyperinsulinemic-euglycemic clamp is performed. However, we believe this is only a theoretical issue. First, in normal, young, glucose-tolerant individuals during the hyperglycemic portion of the clamp, the average insulin levels do not exceed ~200 pmol/l (in older individuals or in Type 2 diabetics, this may be as much as 50% lower). Second, during the recovery portion, plasma insulin levels return to the basal level. Third, the plasma insulin levels during the hyperinsulinemic-euglycemic portion are ~400 pmol/l, which is more than two- to fourfold higher than during the hyperglycemic portion of the clamp. Thus, even if there was a change in sensitivity, due to the endogenous hyperinsulinemia, the high stable exogenous hyperinsulinemia would have such a larger effect that the difference would not be quantifiable even if the two clamps were performed on separate occasions. In fact, in additional studies in our laboratory, the M (glucose utilization) quantified during the euglycemic clamp in both normal and Type 2 diabetics at this dose of hyperinsulinemia are not statistically different when the studies are performed as described above compared with when only a hyperinsulinemic-euglycemic clamp is performed. The use of these multistest paradigms is not uncommon. Investigators who examine both β-cell sensitivity and peripheral tissue sensitivity to insulin in a single setting either perform an intravenous tolerance test immediately followed by a euglycemic clamp (37) or the frequently sampled intravenous glucose tolerance test (Min-mod) where a bolus of insulin is administered 20 min after administration of a bolus of glucose. In the latter test, a bolus of glucose is followed 20 min later either by an injection of tolbutamide or insulin (25). In our new variant of the clamp, hyperglycemia is accurately reproduced in individuals followed by an examination of peripheral tissue sensitivity with a reproducible and steady-state hyperinsulinemia. A relatively moderate increase of plasma glucose (5.4 mmol/l above basal), short duration of infusions (60 min), and careful control of all infusions, the measurement of hepatic glucose production, and a 1-h delay between infusions were employed so that metabolic variables could return to baseline. Therefore, this variant of the clamp does allow assessment of β-cell sensitivity and peripheral tissue sensitivity among different states of glucose tolerance in a single session.

It should be noted the glucose homeostasis comparisons among athletes is cross sectional and not longitudinal. Thus we are assuming that the preservation noted among athletes and the differences between ath-
Let's and controls are due to changes in body composition brought on by long-term vigorous physical activity. It is equally plausible that there is a self-selection bias (parents, peer pressure) or a genetic factor that leads some individuals into a life of vigorous physical activity and some individuals to a sedentary lifestyle. This will in turn result in competitive training, or lack of, which directly alters body composition. The alterations include changes in muscle-to-fat ratios, composition of muscle type, enzymatic sensitivity, as well as changes in the circulating system, including cardio- and pulmonary function, and the capillary density of the muscle bed.

In summary, older female athletes not only prevent the usual decline in insulin sensitivity observed with age but also maintain a similar sensitivity to insulin action as young female athletes. This may be mediated in part through reduced levels of body fat and maintenance of muscle mass. Furthermore, highly trained female athletes have an enhanced glucose uptake and insulin action compared with sedentary women. This suggests that the glucose intolerance normally associated with aging can be prevented by continuation of physical activity and fitness levels that also promote positive adaptations in body composition.

We extend our appreciation to the participants in the study. We also thank Barbara Nicklas for assistance, our physician colleagues for providing medical coverage, and the nurses and laboratory technicians in the Geriatrics Services at the Baltimore Veterans Affairs Medical Center for technical assistance.

This study was supported by funds from National Institutes of Health Grants KO1-AG-00747, 1T32-AG-00219, P60-AG-12583, and AG-00599 and the Department of Veterans Affairs Baltimore Geriatric Research Education and Clinical Center.

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