Effect of age and endurance training on the capacity for epinephrine-stimulated gluconeogenesis in rat hepatocytes

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Submitted 6 December 2002; accepted in final form 25 April 2003

Sumida, Ken D., Steven M. Arimoto, Michael J. Catanzaro, and Frank Frisch. Effect of age and endurance training on the capacity for epinephrine-stimulated gluconeogenesis in rat hepatocytes. J Appl Physiol 95: 712–719, 2003; 10.1152/japplphysiol.01125.2002.—The effects of endurance training on hepatic glucose production (HGP) from lactate were examined in 24-h-fasted young (4 mo) and old (24 mo) male Fischer 344 rats by using the isolated-hepatocyte technique. The liver cells were incubated for 30 min with 5 mM lactate ([U-14C]lactate; 25,000 dpm/ml) and nine different concentrations of epinephrine (Epi). Basal HGP (with lactate only and no Epi) was significantly greater for young trained (T) (99.6 ± 6.2 nmol/mg protein) compared with young controls (C) (72.2 ± 3.9 nmol/mg protein). After the incubation with the various concentrations of Epi, Hanes-Woolf plots were generated to determine kinetic constants (Vmax and EC50). Maximal Epi-stimulated hepatic glucose production (Vmax) was significantly greater for young T (142.5 ± 6.5 nmol/mg protein) compared with young C (110.9 ± 4.8 nmol/mg protein). Similarly, the Vmax was significantly greater for old T (138.2 ± 5.0 nmol/mg protein) compared with old C (103.9 ± 2.5 nmol/mg protein). Finally, there was an increase in the EC50 from the hepatocytes of old T (56.2 ± 6.2 nM) compared with young T (32.6 ± 4.9 nM). In like manner, there was an increase in the EC50 from the hepatocytes of old C (59.7 ± 5.8 nM) compared with young C (33.1 ± 2.7 nM). The results suggest that training elevates HGP in the basal and maximally Epi-stimulated condition, but with age there is a decline in EC50 that is independent of training status.

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THE BRAIN AND NERVE TISSUES depend on the maintenance of blood glucose to sustain a concentration gradient for extraction (16). When liver glycogen stores become depleted, hepatic gluconeogenesis becomes an essential component in an organism’s ability to maintain blood glucose levels. Endurance training has been shown to resist the decrements in blood glucose concentrations during prolonged exercise despite depletion of hepatic glycogen stores (7). Our laboratory has previously demonstrated an enhanced hepatic gluconeogenic capacity attributable to endurance training in rats (7, 32, 33). The augmentation in glucose production ability in the liver could help to account for the maintenance of blood glucose levels in trained animals during prolonged exercise when hepatic glycogen stores become depleted (8). Conversely, recent reports have demonstrated age-related declines in hepatic gluconeogenic capacity (25–27). This loss in liver function could compromise the ability of an elderly individual to respond to a given stress. However, reports from Podolin et al. (25–27) demonstrate that endurance training can help to offset the age-related decline in the liver’s glucose production capacity.

Hormonal stimulation can increase hepatic gluconeogenesis above basal rates. The impact of glucagon (26) and norepinephrine (25) stimulation on hepatic gluconeogenic capacity after endurance training has previously been examined in liver slices from rats. Podolin et al. (26) reported an increase in gluconeogenic capacity in liver slices from a maximal glucagon dose after endurance training that was independent of age. In addition, Podolin et al. observed in young, middle-aged, and old rats a training-induced increase in hepatic glucose production when stimulated with a maximal phenylephrine concentration. Although these observations show promise of the import of endurance training in the improved resistance to hypoglycemia evoked by prolonged exercise, an extrapolation of these observations to the in vivo condition remain controversial. Specifically, circulating levels of glucagon, epinephrine, and norepinephrine are lower in trained individuals during exercise (15, 35). Thus there is skepticism pertaining to the importance of the training-induced adaptations in the liver for the maintenance of blood glucose homeostasis. However, a possible mechanism to account for the lower hormonal milieu in trained individuals could be an adaptation that augments the sensitivity of the liver to various hormones. Although the prior studies by Podolin et al. (25, 26) offer insight into the augmented training-induced response on hepatic gluconeogenesis to hormones, they were limited to an examination on the effects of maximal hormone stimulation where hormone sensitivity was not assessed. Only the observation from Drouin et al. (9) has offered an indication about possible training-induced alterations in hormone sensitivity. In trained subjects, they demonstrated an elevation in resting hepatic glucose output when glucagon and insulin lev-

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els were clamped and maintained at equivalent concentrations compared with controls. They attributed the increase in hepatic glucose output to an increase in glucagon sensitivity (9). In support, Legare et al. (21) later reported an increased density of glucagon receptors in the livers from endurance-trained rats. Barring the report by Drouin et al. (9), little is known about the possible training-induced hepatic alterations to hormone sensitivity. Given that catecholamines play an active role in augmenting precursor availability and can also directly stimulate glucose production, we chose to examine the effects of epinephrine on hepatic gluconeogenic capacity from young and old animals after a program of exercise training.

The purpose of the present study was to assess the impact of epinephrine stimulation on hepatic glucose production from lactate after endurance training from young and old animals. Specifically, with use of the isolated-hepatocyte technique, we sought to determine the following: 1) whether there are any alterations in the sensitivity or the maximal response to epinephrine after endurance training and 2) the impact of age (if any) on the training response to epinephrine. On the basis of previous studies, we hypothesized that, irrespective of age, endurance training would result in an elevation in basal hepatic gluconeogenic capacity and that at maximal hormone concentrations the peak gluconeogenic capacity would be further elevated from the liver cells of endurance-trained animals. We also anticipated that endurance training would result in a greater sensitivity to epinephrine and that age would not prevent a normal response to hormone stimulation or any training-induced adaptation.

METHODS

The experimental protocol for this study was preapproved by the Chapman University Institutional Review Board and conformed with the National Institutes of Health Guide for the Care and Use of Laboratory Animals. Male Fischer 344 rats were obtained from the National Institute on Aging and were either 2 mo (n = 10) or 22 mo (n = 12) of age on arrival. Animals were housed individually in a temperature-controlled room, had free access to food and water, and were kept on a 12:12-h light-dark cycle. After a period of 3 days to allow for continual gassing with 95% O2-5% CO2 while the hindlimb musculature of all animals (soleus, gastrocnemius, plantaris, vastus, gracilis, and biceps femoris) was freeze fixed and stored at −80°C prior to analysis. After the surgical isolation of the liver, the animal was placed in a humidified and temperature-controlled (37°C) Plexiglas perfusion chamber, identical to the perfusion chamber described in detail by Exton and Park (12). Before entering the liver, the perfusate was sequentially filtered through a nylon mesh, oxygenated (95% O2-5% CO2), and then passed through a bubble trap. Hepatocytes were isolated as previously reported by our laboratory (31). Briefly, calcium-free Krebs-Hensleit bicarbonate buffer and fresh thoroughly washed bovine red blood cells (hematocrit of 13–15%) perfused the liver (single pass) for 10 min at a rate of 35 ml/min. The system was switched to a recirculation mode, collagenase was added (30 mg), and the flow rate was adjusted to ~2 ml/min−1·g liver−1. The liver was perfused for 20–30 min to allow for the degradation of connective tissue and visually monitored. The liver was carefully removed from the animal and placed in a petri dish containing an incubation buffer similar to the perfusate but with the addition of calcium (2.4 mM) and the absence of collagenase. Scissors were used to open any remaining intact liver capsules and gently stirred until there was a concentrated homogenate of cells. The cells were then funneled into a 250-ml Nalgene flask, aerated, and shaken in a 37°C water bath for 10 min. The cells were filtered through two layers of a nylon mesh and centrifuged. The supernatant was aspirated and the cells were washed three more times by using an incubation buffer containing 1% (wt/vol) gelatin (Difco). Before the first wash and at the end of the third wash, a small aliquot was tested for viability by using a Trypan blue exclusion test. In addition, another small aliquot was removed to determine the endogenous glucose concentration (before the addition of any substrate) and the initial glycogen content. After the final wash, the cell volume was measured and the cells were reconstituted (14 ml buffer/1 ml cells) in an incubation buffer now containing 1.5% (wt/vol) gelatin (Difco), 5 mM neutralized lactate, and [U-14C]lactate (25,000 dpm/ml). The cell suspensions from a given liver were then separated into 50-ml plastic flasks and attached to a 10-lane manifold that allowed for continual gassing with 95% O2-5% CO2 while the flasks were being gently shaken in a 37°C water bath.

A 10-min preincubation period, to allow for equilibration of lactate and the reestablishment of basal metabolism and ion gradients (1, 6), was employed before the addition of various concentrations of epinephrine (0, 10, 25, 50, 100, 250, 500, 1,000, 5,000, and 10,000 nM) to the cell suspension. The two
highest epinephrine concentrations used were based on a previous study (20), whereas the lower epinephrine concentrations were chosen on the basis of preliminary experiments performed in our laboratory. Once the epinephrine was added, gassing was immediately restored and the cells were incubated with epinephrine for a total of 30 min. This incubation time has previously been demonstrated to be well within steady-state levels of second messengers, enzyme alterations, and gluconeogenesis (6). After the 30-min incubation, reactions were terminated by placing aliquots of the cell suspension into tubes containing perchloric acid, potassium hydroxide, and sodium hydroxide for the subsequent determination of glucose (29) and lactate (17), glycogen (10), and hydroxide, and sodium hydroxide for the subsequent determination of glucose (29) and lactate (17), glycogen (10), and enzyme activity as previously described (32, 33). Skeletal muscle citrate synthase (EC 4.1.3.7) activity was determined by using the procedure of Srere (30).

Calculation and statistics. Hepatic glucose production (HGP) was calculated as the difference between exogenous glucose production (from lactate) and endogenous glucose synthesis (no substrate). For all groups, the glucose production, lactate uptake, and glycogen content were divided by the cell viability and expressed as the concentration (nmol or, where applicable, dpm) per milligram of protein. [14C]glucose production (dpm/ml) was the radioactive glucose appearance in the incubation medium normalized to a lactate-specific activity of 5,000 dpm/μmol and expressed as dpm per milligram of protein. Dose-response curves were generated, and Hanes-Woolf plots were used to determine the maximal epinephrine response (V_{max}) and half-maximal effect (EC_{50}), which denotes epinephrine sensitivity. Differences between the regressions generated from Hanes-Woolf plots were determined according to the procedures of Zar (36). For all other comparisons, a two-way (age vs. training status) ANOVA was employed. A significant F ratio was analyzed by using a Student-Newman-Keuls post hoc test. The level of significance was set at P < 0.05, and the values are expressed as means ± SE.

## RESULTS

The body weights between control and trained young animals were not significantly different after the 24-h fast (Table 1). Similarly, the body weight for the old animals was not significantly different between control and trained groups after the 24-h fast (Table 1). As a reflection of age, the body weight for the young animals (231.5 ± 4.2 g) was significantly lower than for the old animals (369.7 ± 6.1 g) after the 24-h fast. To support the effectiveness of the training regimen, the skeletal muscle citrate synthase activities were significantly greater for trained vs. control animals for both age groups (Table 1).

After the hepatocyte incubation, the pH of the cell suspensions did not significantly differ between young and old animals, nor did it differ between trained and control groups (Table 2). In addition, the protein content from the cell suspensions was not significantly different between young or old animals or between groups (Table 2). The initial lactate concentration for each hepatocyte suspension did not vary for any of the groups (Table 2). The initial glycogen content in the liver cells before the addition of lactate was not significantly different between young and control groups (Table 2). Further, there was no significant difference in postglycogen content after the incubation with each epinephrine concentration compared with the initial glycogen content within any of the groups (for brevity, the results of the postglycogen content at the highest epinephrine incubation, i.e., 10,000 nM, are presented in Table 2). There was no difference in cell viability between the prewash and final wash procedure within a group (for brevity, the results from the final wash are reported; Table 2). In contrast, the cell viability was significantly greater from young (88.5 ± 1.2%) compared with old

### Table 1. Animal body weights and enzyme index of training

<table>
<thead>
<tr>
<th></th>
<th>Body Weight, g</th>
<th>Citrate Synthase, μmol·min⁻¹·g⁻¹</th>
</tr>
</thead>
<tbody>
<tr>
<td>Young</td>
<td></td>
<td></td>
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<tr>
<td>Control</td>
<td>237.4 ± 3.1</td>
<td>35.49 ± 4.81</td>
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<tr>
<td>Trained</td>
<td>225.6 ± 7.2</td>
<td>58.51 ± 5.82</td>
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<tr>
<td>Old</td>
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<tr>
<td>Control</td>
<td>378.7 ± 7.2*</td>
<td>22.72 ± 2.61</td>
</tr>
<tr>
<td>Trained</td>
<td>359.2 ± 8.9*</td>
<td>37.58 ± 5.18*</td>
</tr>
</tbody>
</table>

Values are means ± SE, n, no. of rats. *Significantly different from young counterparts, P < 0.05. †Significantly different from control, P < 0.05.

### Table 2. Characteristics of incubation medium and isolated hepatocytes

<table>
<thead>
<tr>
<th></th>
<th>pH</th>
<th>Viability, %</th>
<th>Initial [Lactate], mM</th>
<th>Pre-[Gly]</th>
<th>Post-[Gly]</th>
<th>Protein, mg/ml</th>
</tr>
</thead>
<tbody>
<tr>
<td>Young</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>7.42 ± 0.02</td>
<td>88.2 ± 2.1</td>
<td>5.23 ± 0.02</td>
<td>6.5 ± 1.1</td>
<td>5.1 ± 0.7</td>
<td>7.24 ± 0.57</td>
</tr>
<tr>
<td>Trained</td>
<td>7.42 ± 0.03</td>
<td>88.7 ± 1.6</td>
<td>5.21 ± 0.05</td>
<td>5.0 ± 1.0</td>
<td>3.7 ± 0.6</td>
<td>6.72 ± 0.56</td>
</tr>
<tr>
<td>Old</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>7.39 ± 0.01</td>
<td>69.1 ± 1.5</td>
<td>5.21 ± 0.02</td>
<td>5.4 ± 0.6</td>
<td>5.1 ± 0.8</td>
<td>6.56 ± 0.63</td>
</tr>
<tr>
<td>Trained</td>
<td>7.41 ± 0.01</td>
<td>68.4 ± 1.9*</td>
<td>5.15 ± 0.02</td>
<td>6.4 ± 0.7</td>
<td>5.4 ± 0.4</td>
<td>6.48 ± 0.59</td>
</tr>
</tbody>
</table>

Values are means ± SE, n, no. of rats. From the Trypan blue exclusion test, the prewash viability did not differ from the final wash viability. Viability (%) above reflects final wash value. Initial glycogen concentration (Pre-[Gly]) is the content before the addition of epinephrine. Final glycogen concentration (Post-[Gly]) is the content after maximal epinephrine stimulation. All glycogen concentrations are divided by the protein content and reflect 100% cell viability (see METHODS section). *Significantly different from young counterparts, P < 0.05.
animals (68.8 ± 1.1%) but was unaffected by training status (Table 2).

Although the [14C]glucose data mirrored the relationships observed for glucose, for clarity, the major focus will be the nonradioactive data because the radioactive data merely serve to support the glucose production from the liver. Furthermore, the kinetic analyses (Hanes-Woolf plots) were determined from the nonradioactive data. In this regard, age did not affect the basal gluconeogenic capacity within a group (i.e., control vs. trained). Adjustment of the gluconeogenic rates to reflect 100% cell viability resulted in no difference between young (78.2 ± 6.0 nmol/mg protein) and old control animals (72.2 ± 3.9 nmol/mg protein) when incubation was with lactate only and in the absence of epinephrine. Similarly, basal HGP after the adjustment to reflect 100% cell viability failed to reveal differences between young (99.6 ± 6.2 nmol/mg protein) and old control animals (97.3 ± 5.9 nmol/mg protein) when incubation was with lactate only and in the absence of epinephrine. Although age did not affect the basal gluconeogenic capacity within control or trained groups, the basal HGP was significantly greater for trained animals compared with controls in both young and old animals (Fig. 1). In support, the adjusted lactate uptake (reflecting 100% cell viability) was greater in trained compared with control hepatocytes in both young and old animals (Fig. 2). Furthermore, the basal [14C]glucose production (Fig. 2) was also significantly greater in young and old trained animals (483.1 ± 32.0 dpm/mg protein) vs. controls (377.2 ± 22.7 dpm/mg protein).

Epinephrine-stimulated HGP demonstrated saturation kinetics in both trained and control groups (Fig. 1). Training elevated the maximal response to epinephrine in both young and old animals (Fig. 1). Hanes-Woolf plots demonstrated significantly different slopes for trained vs. control animals in both young and old groups (Fig. 3). As such, endurance training significantly elevated the basal HGP, and this elevation was maintained at all epinephrine concentrations. In support, maximal epinephrine-stimulated [14C]glucose production (Fig. 2) was significantly greater in trained animals (682.9 ± 43.2 dpm/mg protein) vs. controls (541.2 ± 23.8 dpm/mg protein). Although training status elevated the maximal response, it did not affect the EC50 within a group (i.e., young or old). In contrast, whereas age did not have any impact on maximal hormone stimulation within a group, it significantly elevated the half-maximal effect, which was independent of the training status (Table 3).

**DISCUSSION**

In contrast to prior studies (25–27), but in support of others (20, 31), there was no age-related decline in...
basal gluconeogenic capacity from lactate, nor was there any change in the maximal response to epinephrine. That the glucose production reflects gluconeogenic capacity is supported by the depletion of hepatic glycogen after the 24-h fast, the minimal glycogenolysis after the incubation with lactate, and the [14C]glucose production. As it pertains to chronic exercise, there was a training-induced increase in the basal hepatic gluconeogenic capacity. As before, this is supported by the depletion of hepatic glycogen, the minimal glycogenolysis, and the significant increases in [14C]glucose production and lactate uptake. Furthermore, the training-induced increase in glucose production was maintained in response to epinephrine. Finally, age did not affect the training-induced increase in basal hepatic glucose production, nor did it change the liver’s ability to respond to maximal epinephrine stimulation; however, senescence lowered the EC50, and training did not prevent the decline in epinephrine sensitivity.

Endurance training has been associated with an enhanced ability to resist the decrements in blood glucose levels during exercise (4, 7) despite lower concentrations of glucagon, epinephrine, and norepinephrine (15). An elevation in basal glucose production capacity after endurance training has previously been observed in rats with the use of the perfused liver technique (32, 33) and perfused hepatocytes (5). This study supports the training-induced increase in basal hepatic gluconeogenesis with use of isolated hepatocytes. In addition, several studies have reported elevations in liver glucose production capacity in response to various hormones after endurance training (9, 25, 26). Drouin et al. (9) observed an increase in hepatic glucose production response in trained subjects vs. controls when glucagon and insulin were clamped at equivalent levels. They attributed the increase in glucose appearance to be due to an elevation in glucagon sensitivity on hepatic glycogenolysis (9). In support, Legare et al. (21) examined the binding properties of glucagon receptors in plasma membranes isolated from the livers of trained vs. control animals. They reported an increase in liver glucagon-receptor density from endurance-trained rats. Nieto et al. (24) also observed an adaptive response to endurance exercise with the use of rat liver plasma membranes. They reported that endurance exercise enhanced the adenyl cyclase system via the stimulation of the enzyme catalytic subunit and the stimulatory G protein (24). This observation has recently been supported by Podolin et al. (28), who demonstrated that endurance-training resulted in greater binding affinity for glucagon receptors from the livers of trained animals compared with sedentary counterparts. Given the training-induced increase in glucagon receptor density and the efficacy in stimulating the adenyl cyclase system, it remains a possibility that other hormones (i.e., catecholamines) could similarly augment the liver’s capacity for glucose production after a chronic program of endurance training. Collectively, this could explain the lower hormonal milieu observed in trained individuals (15).

This potential has been partially addressed by Podolin et al. (25), who demonstrated significant elevations in gluconeogenic capacity in liver slices from trained animals compared with controls from various concentrations of norepinephrine. In their incubation medium, they also included glucagon and insulin. In this regard, it is possible that the combination of norepinephrine and the other hormones is required to elicit an effect. In a subsequent report, Podolin et al. (26) specifically examined the effects of training with use of three distinct gluconeogenic stimulators: glucagon, phenylephrine (α-adrenergic agonist), and isoproterenol (β-adrenergic agonist). They observed greater hepatic glucose production in response to maximal levels of glucagon and phenylephrine in liver slices from trained vs. control rats (26). However, they failed to observe any training effect from isoproterenol (26). Whereas catecholamines can stimulate both α- and β-adrenergic receptors, it is generally agreed that the control of glucose production by epinephrine in the liver is primarily mediated by α1-receptors (23).

Table 3. Kinetic parameters determined by Hanes-Woolf analysis

<table>
<thead>
<tr>
<th></th>
<th>n</th>
<th>EC50, nM</th>
<th>Vmax, nmol/mg protein</th>
</tr>
</thead>
<tbody>
<tr>
<td>Young</td>
<td></td>
<td>33.1 ± 2.7</td>
<td>110.9 ± 4.8</td>
</tr>
<tr>
<td>Control</td>
<td>5</td>
<td>32.6 ± 4.9</td>
<td>142.4 ± 6.5†</td>
</tr>
<tr>
<td>Old</td>
<td></td>
<td>59.7 ± 5.8*</td>
<td>103.9 ± 2.5</td>
</tr>
<tr>
<td>Trained</td>
<td>6</td>
<td>56.2 ± 6.2*</td>
<td>138.2 ± 5.0†</td>
</tr>
</tbody>
</table>

Values are means ± SE; n, no. of rats. EC50, concentration of epinephrine (nM) required to elicit one-half the maximal epinephrine stimulation. Vmax, maximal epinephrine stimulation expressed as nmol of glucose produced from lactate divided by the protein content and reflects 100% cell viability (see METHODS). *Significantly different from young counterparts, P < 0.05. †Significantly different from control, P < 0.05.
such, that endurance training resulted in an elevation in $V_{\text{max}}$ from epinephrine compared with controls in the present study is consistent with the findings of Podolin et al. However, we also observed no alteration in the EC$_{50}$. This observation was in contrast to our hypothesis. Nevertheless, Mazzeo et al. (22) observed that endurance training had no effect on liver β-receptor number or affininity in rats. Whether there is a similar absence in hepatic α$_1$-receptor affinity or maximal binding number after training has yet to be determined. In like manner, whether the training-induced epinephrine responsiveness in the liver is attributable to changes in second-messenger signaling and/or response is unknown.

The preservation of basal hepatic gluconeogenic capacity with age is consistent with prior reports (20, 31). In addition, our laboratory previously observed the maintenance of hepatic glucose production rates in hepatocytes from young and old animals with the use of various precursors entering the gluconeogenic pathway at distinct sites (31). However, this observation is in contrast to the studies of Podolin et al. (25–27), where they consistently reported age-related declines in glucose production capacity with the use of liver slices. An explanation for this discrepancy remains to be elucidated, but it is conceivable that the age-related decline in cell viability, as observed in the present study, represents an overall loss in organ function (at any given time there are less viable cells) and/or the fragile integrity of older hepatocytes that is exacerbated with the use of liver slices (a technique that can result in further damage to cells). When the cell viability is normalized for all groups (i.e., corrected to 100% viability) the hepatic gluconeogenic capacity is maintained with age. In addition, the finding that the remaining viable cells from the old animals demonstrate saturation kinetics to epinephrine suggests that these cells are able to maintain their integrity.

In the present study, maximal epinephrine-stimulated glucose production was also unaltered with age. This observation is consistent with Kmiec and Myśliwski (20), who reported similar gluconeogenic rates from young and old rat hepatocytes incubated with 18 mM lactate and 2 mM pyruvate and stimulated with maximal epinephrine concentrations. They also observed no age-related decline in the gluconeogenic response with maximal norepinephrine levels (20). We have extended the observation to include an increase in the EC$_{50}$ that suggests an age-related alteration in the sensitivity to epinephrine (irrespective of training status). Furthermore, the loss in epinephrine sensitivity attributable to age may in fact be greater than we report because at any given epinephrine concentration there are fewer viable cells from old compared with young animals. Borst et al. (2) previously reported that α$_1$-receptor density, expressed as binding sites per cell, is unchanged with senescence. In addition, they observed that epinephrine response and sensitivity, as determined by phosphoinositide hydrolysis, was also unchanged with age (2). Although the preservation of maximal epinephrine response with advanced age is consistent with Borst et al. (2), the discrepancy as it pertains to sensitivity remains unknown, but it does support the contention that senescence retards the metabolic response to a given stress. The decline in the EC$_{50}$ may be related to events distal to phosphoinositide hydrolysis (i.e., an alteration in calcium release, calmodulin, and/or enzyme phosphorylation) that can eventually be obviated, but only at maximal hormone concentrations.

Although we recognize the caution in extrapolating the present results to in vivo conditions, our findings are consistent with the lower catecholamine levels observed in trained individuals during exercise (15, 35). Specifically, our results indicate that the maximal epinephrine-stimulated glucose production from controls was only ~10% higher than what is observed at basal from trained livers in both young and old animals. Under these conditions, trained individuals could secrete substantially less epinephrine to evoke the same hepatic response for gluconeogenesis. An additional interpretation of our results involves the following. In both humans (14) and rats (4, 7), in vivo glucose production is tightly regulated to support glucose disposal so that euglycemia is maintained. In skeletal muscle, epinephrine stimulation has recently been observed to increase glycogenolysis and glycolysis but to decrease glucose disposal (34). In addition, Mazzeo et al. (22) did not observe any change in β-adrenergic receptor characteristics in the soleus muscle after endurance training in rats. Taken together, these observations suggest that the lower catecholamine secretion in trained individuals would attenuate skeletal muscle glycogenolysis and elevate glucose disposal. The training-induced sparing of skeletal muscle glycogen during exercise is a consistent observation (18). Conversely, the potential increase in skeletal muscle glucose disposal could be tempered by a training-induced increase in the use of free fatty acids from adipose tissue (11) and intramuscular triglycerides (19). However, because the exercise is prolonged resulting in a depletion of muscle and eventually liver glycogen, blood glucose utilization would elevate. Catecholamine secretion would now be augmented, which increases gluconeogenic precursor availability (35), and the training-induced elevation in hepatic glucose production capacity would now play a pivotal role in the maintenance of euglycemia. Thus our results suggest that, after training, the increased capacity for epinephrine-stimulated liver gluconeogenesis is propitious for the maintenance of euglycemia during exercise specifically when blood glucose homestasis is markedly challenged. The fact that a senescent liver retains its capability to adapt to endurance training would be efficacious for glucose homestasis and help to counteract other age-related declines associated with glucose metabolism.

Finally, we note the age-related decline in liver cell viability. It is unknown whether the lower cell viability from the old animals is an inherent loss in organ function (again, at any given time there are less viable cells) or whether it is another aspect of the isolation procedure we employed that compromises the integrity
of senescent cells. However, the same solutions were used on both young and old hepatocytes on a given day, yet the cell viability from the young animals was greater than their older counterparts. In addition, the loss of cell viability from old animals was not due to the washing procedure (the protocol where the greatest damage to cells can occur). We have no explanation for the difference in cell viability between young and old animals and can only speculate that the perfusate flow rate for the old animals may have been a factor. We employed a common procedure using a rapid washout (initial perfusate flow of 35 ml/min) and then established a flow of ~2 ml·min⁻¹·g liver⁻¹ to ensure adequate oxygenation during the perfusion with collagenase. Because the livers from old animals were significantly larger (due to their size) this required a greater absolute flow rate. It is possible that this constant high flow rate from the rapid washout and perfusion with collagenase may have inadvertently damaged the cells from old animals. Alternatively, if we had lowered the absolute flow during the perfusion with collagenase, then inadequate oxygenation could have similarly damaged the cells. We know of only one study (13) that examined the effect was elevated in the hepatocytes from old compared with young animals, irrespective of training status. Thus, whereas the hepatic gluconeogenic capacity in response to endurance training and maximal epinephrine stimulation is maintained with advanced age, exercise training has no effect on epinephrine sensitivity.

In summary, the present results indicate that basal glucose production capacity from lactate is augmented in the liver after endurance training and that the hepatic training adaptation is not lost with age. In addition, the enhanced hepatic gluconeogenic capacity after training is maintained at all epinephrine concentrations. Although endurance training elevated the basal (no epinephrine) and maximal epinephrine response, it did not alter the EC₅₀. Furthermore, basal glucose production capacity did not decline with advanced age. Senescence did not alter the maximal epinephrine response; however, the half-maximal effect was elevated in the hepatocytes from old compared with young animals, irrespective of training status. Thus, whereas the hepatic gluconeogenic capacity in response to endurance training and maximal epinephrine stimulation is maintained with advanced age, exercise training has no effect on epinephrine sensitivity.

The authors thank Suzanne Crandall, David Frampton, Todd Van Etten, and Eric Hanson for valuable assistance in the training of animals and assistance in the collection of data. In addition, we express special thanks to Dr. Timothy Chan for the hepatocyte isolation procedure and continual support and guidance.

DISCLOSURES

This study was supported by National Institute on Aging Grant AG-14565-01.

REFERENCES


