Alterations in Key Gluconeogenic Regulators With Age and Endurance Training

Deborah B. Horn, Deborah A. Podolin, Jacob E. Friedman, David A. Scholnick, and Robert S. Mazzeo

The purpose of the present investigation was to examine changes in six potential regulators of hepatic gluconeogenesis with normal aging and endurance training: fructose 2,6-bisphosphate (F 2,6-P_2), mitochondrial and cytosolic phosphoenolpyruvate carboxykinase (PEPCK) activity, PEPCK mRNA, and pyruvate carboxylase and malate dehydrogenase activity. Young (4 months), middle-aged (12 months), and old (22 months) male Fischer 344 rats (N = 66) were divided into trained and sedentary groups. Trained animals were run 1 h/d, 5 d/wk for 10 weeks at treadmill speeds of 75% age-specific maximal running capacity. Animals were killed at rest, and the right main lobe of the liver was removed. F 2,6-P_2 levels were significantly greater in old compared with young animals regardless of training condition (119% and 80% increase in old trained and untrained animals, respectively). No changes were found with training. Rates of cytosolic PEPCK activity declined significantly with age in both trained (1.3 ± 0.1, 1.0 ± 0.1, and 0.7 ± 0.1 μmol/g/min in young, middle-aged, and old, respectively) and untrained (1.3 ± 0.1, 1.1 ± 0.1, and 0.8 ± 0.2 μmol/g/min) groups. Training did not result in any significant differences between age groups. PEPCK gene expression (mRNA) determined by Northern blot analysis decreased 30% in trained and untrained old animals compared to the young counterparts; again, training had no effect in any age group. No significant differences were found in pyruvate carboxylase, mitochondrial PEPCK, or malate dehydrogenase activity with either age or training. These results suggest that previous age-related declines found in hepatic gluconeogenic capacity can be attributed, in part, to changes in F 2,6-P_2, cytosolic PEPCK activity, and PEPCK mRNA, but not to alterations in the activities of mitochondrial PEPCK, malate dehydrogenase, or pyruvate carboxylase. Since training had no effect on any regulator studied, the factors responsible for attenuation in the age-related decline in gluconeogenesis with training remain to be determined.

OUR GROUP AND OTHERS have previously documented age-dependent alterations in gluconeogenesis.[1-5] This becomes increasingly important when liver glycogen stores begin to deplete (ie, during starvation and prolonged exercise), since gluconeogenesis is essential to blood glucose maintenance via hepatic glucose output under these conditions.[6,7] However, the mechanism responsible for this age-related observation and the extent to which endurance training can be an effective intervention remain unclear.

For gluconeogenesis to occur, three energetically irreversible steps in glycolysis must be bypassed. The enzymes used to bypass these irreversible reactions are glucose 6-phosphatase, fructose 1,6-bisphosphatase, pyruvate carboxylase, malate dehydrogenase, and phosphoenolpyruvate carboxykinase (PEPCK). PEPCK is considered the rate-limiting enzyme in gluconeogenesis and is thought to be regulated primarily through changes in transcription.[8] Another important regulator of gluconeogenesis is the metabolite fructose 2,6-bisphosphate (F 2,6-P_2). Elevated levels of F 2,6-P_2 have been found to stimulate phosphofructokinase and inhibit fructose 1,6-bisphosphatase, thereby inhibiting gluconeogenesis overall.[9]

Age-related declines in gluconeogenesis can partially account for the impaired capacity to maintain blood glucose homeostasis with age under conditions known to elicit hypoglycemia. Studies from our laboratory have shown that declines in gluconeogenic capacity occur with advancing age in the rat.[3-5]

In contrast to the effects of aging, endurance training has been shown to enhance hepatic gluconeogenic capacity.[3-5,7,10,11] However, specific enzyme activities within the gluconeogenic pathway have been examined and were found to be unchanged with training. Podolin et al[3-5] measured hepatic glycogen synthase[3] and cytosolic PEPCK activity and found no significant differences between trained and untrained animals. Fructose 1,6-bisphosphatase activity has also been reported to be unaltered by exercise training.[10,12] These findings suggest that the exercised-induced alterations in gluconeogenesis likely occur below the level of the triose phosphates. Consequently, it was the purpose of the present study to examine the following sites of gluconeogenic regulation in response to aging and endurance training: F 2,6-P_2 content and the activities of mitochondrial and cytosolic PEPCK, malate dehydrogenase, and pyruvate carboxylase. It was our hypothesis that changes in some of these key regulators may partially account for declines observed in gluconeogenesis with age and contribute to training effects observed in this pathway.

MATERIALS AND METHODS

Animals

Sixty-six male Fischer 344 rats (Harlan Sprague-Dawley, Indianapolis, IN) were used: 22 young, 22 middle-aged, and 22 old animals. Animal ages upon arrival were 4, 12, and 22 months. Animals were housed in pairs at 24°C with a 12-hour light/dark cycle and given Teklad (Bartonville, IL) rodent chow and water ad libitum. Before experimentation, all animal procedures were approved by the University of Colorado Institutional Animal Care and Use Committee.

Initial Testing

After a 1-week acclimatization period, a graded exercise test (GXT) was administered to each rat on a 10-lane motorized treadmill. The test was used to determine maximal running speed and initial levels of fitness for each age group.[13] Based on the results of this test, animals were pair-matched and then randomly assigned to the trained or untrained group.

Training Protocol

The 10-week endurance training protocol consisted of running five times per week up a 15% grade at a speed that elicited 75% of the

From the Department of Kinesiology, University of Colorado, Boulder, CO: and the Nutrition Department, Case Western Reserve University, Cleveland, OH.

Submitted July 10, 1996; accepted October 31, 1996.

Address reprint requests to Robert S. Mazzeo, PhD, Box 354, Department of Kinesiology, University of Colorado, Boulder, CO 80309-0354.

0026-0495/97/4604-0014$03.00/0

animal's maximal capacity as determined from the initial GXT.[6-8] The duration of the training sessions, initially 10 min/d, was increased by 5 min/d until a total running time of 60 minutes was achieved. Following the fifth week, the speed was increased 3 m/min and the duration kept constant until the training period was complete.[13]

The untrained group ran 1 day every other week for 5 minutes at 75% of their predetermined maximal capacity. This was to maintain familiarization with treadmill running and investigator handling.

After the training period, all animals performed a second GXT to determine final maximal running capacity. Three days after this GXT, all animals completed an endurance test. The animals were run at 75% of pretrained maximal capacity to exhaustion, which was defined as inability to avoid the shock grid or loss of righting reflex.[12] Confirmation of a training effect was made by measuring citrate synthase activity in the soleus muscle by the method used by Srere.[14]

Tissue Preparation

Animals were killed at least 3 days after the last exercise bout at the ages of 7 months (young), 15 months (middle-aged), and 25 months (old). The animals were fasted overnight to reduce liver glycogen levels and facilitate measurement of maximal gluconeogenic capacity.

The animals were anesthetized (intraperitoneally, pentobarbital 60 mg/kg body weight). The right main liver lobe was excised and homogenized as described by Gohil et al.[15] Liver samples were diluted 10 times with 250 mmol/L sucrose buffer. Homogenization consisted of forceful compression with a Pyrex #7727 (Corning, NY) homogenizer followed by three manual passes. The buffer was allowed to resaturate the tissue, and the compression–three-pass treatment was repeated. The supernatant was poured off and centrifuged at 850 × *g* (2,700 rpm) for 5 minutes using a Beckman (Palo Alto, CA) high-speed centrifuge. The supernatant was centrifuged again at 14,500 × *g* (11,250 rpm) for 10 minutes.

The remaining supernatant was frozen at −70°C for analysis of cytosolic PEPCK. The pellet, containing the mitochondrial fraction, was resuspended in 200 µL sucrose buffer and frozen at −70°C for analysis of mitochondrial PEPCK, pyruvate carboxylase, and malate dehydrogenase. For mRNA and F 2,6-P_2 analysis, sections of the right main lobe were removed and immediately frozen in liquid nitrogen for later analysis.

Biochemical Analysis

F 2,6-P_2. Liver F 2,6-P_2 concentrations were determined in neutralized 0.05N NaOH extracts at 32°C by a modified method of Van Schaftingen.[16] Samples of frozen liver were quickly weighed, ground in liquid nitrogen, and then homogenized in 0.05N NaOH (pH > 8.0) to prevent hydrolysis of F 2,6-P_2 by weak acid. Pyrophosphate-dependent phosphofructokinase from potato tubers was coupled with aldolase, triosphosphate isomerase, and alpha-glycerophosphate dehydrogenase reactions. Change in optical density was measured at 340 nm for 5 minutes.

PEPCK. Both cytosolic and mitochondrial PEPCK activity were analyzed using the method developed by Petrescu et al.[17] Briefly, PEPCK activity was measured using deoxyguanosine 5′-diphosphate as the nucleotide substrate to form oxaloacetate. The oxidation of NADH is equal to the rate of oxaloacetate formation, and thus PEPCK activity is indirectly measured.

PEPCK mRNA. Total RNA was isolated from rat liver using the guanidine thiocyanate method as previously described.[18] Concentration and purity of the RNA was assessed by measuring absorbance at 260 and 280 nm. For Northern blot analysis, 20 µg total RNA was mixed with an ethidium-bromide solution and size-fractionated gel. The RNA was transferred to a nylon membrane, cross-linked by UV irradiation, and prehybridized at 65°C for 2 hours in a solution containing 1% bovine serum albumin, 0.5 mol/L EDTA, 0.1 mol/L Na_2HPO_4, 0.7% sodium dodecyl sulfate (SDS), and 1.7% H_3PO_4. The prehybridization was then replaced with a similar solution containing a nick-translated ^{32}P-labeled cDNA probe (10^6 dpm/µg) for PEPCK, or actin. After hybridization overnight at 65°C, the filter was washed extensively in 2× SSC/0.1% SDS at room temperature and dried. The hybridization intensity of Northern blots was quantified with a phosphorimager (Molecular Dynamics, Sunnyvale, CA). The abundance of PEPCK mRNA was determined and expressed relative to actin mRNA to account for differences in mRNA loading.

All DNA-modifying enzymes, radiochemicals, and chemicals were purchased from Boehringer (Indianapolis, IN), Dupont-New England Nuclear (Boston, MA), and Sigma Chemical (St Louis, MO). The cDNA probe (PEPCK) used for hybridization was a 1.1-kb *PstI-PstI* fragment from the 3′ end of the PEPCK cDNA, as described previously.[18] Actin was hybridized using mouse actin cDNA.[19] All cDNA probes were radiolabeled by random priming using dATP, dGTP, dTTP, and [^{32}P]dCTP supplied in a labeling kit obtained from Boehringer.

Pyruvate carboxylase. Mitochondrial pyruvate carboxylase activity was measured by the method used by Crabtree et al.[20] In this assay, acetyl coenzyme A reacts with the indicator dye, DTNB, indicating CO_2 consumption and thus indirectly measuring pyruvate carboxylase activity.

Malate dehydrogenase. Malate dehydrogenase activity determination was based on the method of Englard and Siegel.[21] Mitochondrial malate dehydrogenase converts oxaloacetate to malate with the simultaneous oxidation of NADH.

Statistical Analysis

Enzyme activities, metabolite concentrations, and mRNA levels were analyzed in duplicate and reported as the mean ± SE. Differences were identified with a 2 (trained *v* untrained) × 3 (age group) factorial ANOVA ($P < .05$). Tukey post hoc tests were used to identify significant differences.

RESULTS

Effects of Training on Animal Characteristics

Treadmill running resulted in significantly decreased body weight in the trained versus untrained for both young and middle-aged animals ($P < .05$). Young and middle-aged trained rats exhibited a 12% decrease in body weight compared with age-matched controls. Body weight was also significantly different in response to aging. Middle-aged and old trained rats had significantly greater body weight compared with young animals (Table 1).

Trained animals exhibited significantly increased muscle citrate synthase activity, increased maximum running speed, and increased endurance time across all age groups compared with untrained animals (Table 1). Both trained and untrained animals demonstrated an aging effect for all of these variables.

Enzyme Activity

No differences in mitochondrial PEPCK were observed as a function of age or training status (Table 2). Cytosolic PEPCK activity declined significantly with age in trained (22% and 45% for middle-aged and old, respectively) and untrained (13% and 39%) animals compared with the young (Table 2). No significant differences in cytosolic PEPCK were found in trained versus untrained animals of any age group.

Hepatic pyruvate carboxylase activity declined significantly in old trained animals compared with young and middle-aged trained rats (Table 2). No change was found in the untrained

Table 1. Final Animal Characteristics

Group	No. of Animals	Body Weight (g)	Maximum Running Speed (m/min)	Soleus Citrate Synthase (μmol/g/min)	Endurance Time (min)
Young					
Untrained	12	371.8 ± 4.7	37.7 ± 2.3	29.2 ± 3.0	28.8 ± 2.0
Trained	10	331.1 ± 5.4‡	57.4 ± 2.5‡	43.9 ± 4.3‡	141.9 ± 6.0‡
Middle-aged					
Untrained	12	431.8 ± 5.9*	29.3 ± 1.0*	26.1 ± 1.3	25.1 ± 1.7
Trained	10	384.2 ± 4.4*‡	44.6 ± 1.1*‡	31.1 ± 1.3*‡	136.2 ± 5.5‡
Old					
Untrained	7	377.0 ± 25.8†	19.3 ± 1.0*†	16.2 ± 1.1*	11.0 ± 2.1*†
Trained	8	367.9 ± 17.9*	29.5 ± 2.5*†‡	21.2 ± 1.2*‡	126.3 ± 12.5‡

NOTE. Values are the mean ± SEM.

*Significantly different *v* young animals of comparable training status ($P < .05$).

†Significantly different *v* middle-aged animals of comparable training status ($P < .05$).

‡Significantly different from untrained age cohort ($P < .05$).

group. Pyruvate carboxylase activity for age-matched trained versus untrained animals was not significantly different.

No significant differences were found in malate dehydrogenase activity between young, middle-aged, and old animals. No significant training effect was found between age-matched trained and untrained animals.

PEPCK Gene Expression—mRNA

Age resulted in significantly lower hepatic PEPCK mRNA levels in both trained (30%) and untrained (45%) old animals compared with the young counterparts (Fig 1). Training had no effect on PEPCK gene expression in any age group.

F 2,6-P_2

In both trained and untrained old animals, F 2,6-P_2 levels increased significantly (119% and 80%) compared with the levels in young controls (Fig 2). There was a trend for increasing F 2,6-P_2 levels in both middle-aged trained and untrained animals with advancing age, although only old animals were significantly different from young animals. Training did not affect metabolite levels.

DISCUSSION

Gluconeogenesis, as measured by the rate of incorporation of various precursors such as lactate and fructose into glucose and glycogen, has been shown to decrease with advancing age.[3-5] Recently, our laboratory reported that rates of lactate incorporation into glucose decreased 34% and 63% in old trained and untrained rats compared with corresponding young animals.[5]

Results from a number of investigations have shown that it is possible to increase gluconeogenic capacity with prolonged endurance training.[10,22,23] The efficacy of training as an intervention to age-related declines in gluconeogenesis has been examined. We have previously reported increased lactate incorporation into glucose with 10 weeks of endurance training in young, middle-aged, and old rats compared with untrained animals.[6,8] When fructose was the gluconeogenic precursor,[4] no training effect on the incorporation rate was found. It was suggested that alterations in gluconeogenesis may be occurring in the gluconeogenic pathway between the entrance points of these two substrates. Thus, it was our intention to examine alterations in key regulators located prior to the triose phosphates.

A major finding of this study was that levels of F 2,6-P_2, known to allosterically stimulate phosphofructokinase (and

Table 2. Hepatic Enzyme Activity

Group	Pyruvate Carboxylase	Cytosolic PEPCK	Mitochondrial PEPCK	Malate Dehydrogenase
Young				
Untrained	23.7 ± 1.4	1.3 ± 0.1	1.0 ± 0.2	454.2 ± 15.0
Trained	25.7 ± 1.9	1.3 ± 0.1	0.8 ± 0.2	436.1 ± 18.6
Middle-aged				
Untrained	23.0 ± 1.7	1.1 ± 0.1*	0.9 ± 0.1	430.9 ± 18.3
Trained	23.8 ± 1.7	1.0 ± 0.1*	1.0 ± 0.2	436.1 ± 8.8
Old				
Untrained	23.9 ± 2.3	0.8 ± 0.2*†	1.3 ± 0.2	477.4 ± 19.5
Trained	15.0 ± 1.5*†	0.7 ± 0.1*†	0.9 ± 0.2	390.6 ± 37.1

NOTE. Values are the mean ± SE expressed in μmol/g/min.

*Significantly different *v* young animals of comparable training status ($P < .05$).

†Significantly different *v* middle-aged animals of comparable training status ($P < .05$).

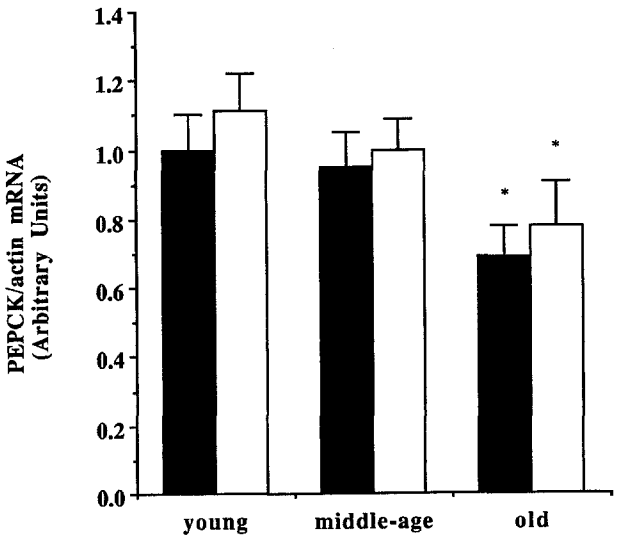

Fig 1. Influence of age and endurance training on hepatic PEPCK mRNA levels in (■) untrained and (□) trained Fischer 344 rats. *Significantly different *v* young animals of comparable training condition ($P < .05$).

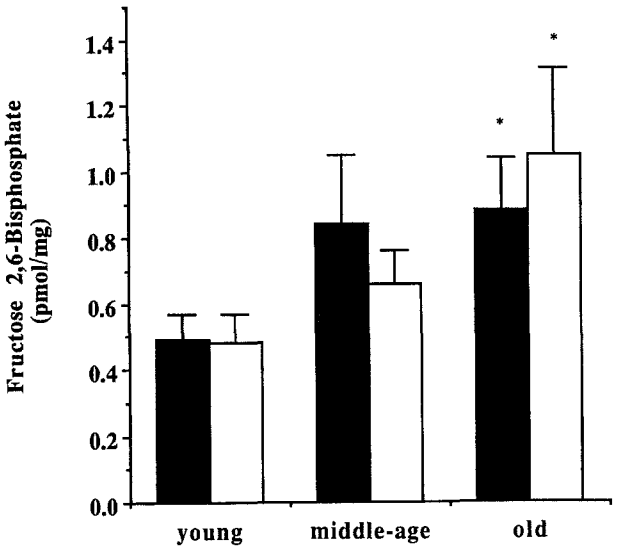

Fig 2. Hepatic F 2,6-P_2 levels as a function of both age and endurance training. *Significantly different *v* young animals of comparable training condition ($P < .05$). (■) untrained; (□) trained.

hence glycolysis) and inhibit gluconeogenesis in liver, were elevated in old compared with young animals. Previous studies have shown that hepatic levels of F 2,6-P_2 are affected by changes in substrate availability and plasma hormone levels.[24-27] In this study, the effects of fluctuating hormone and substrate concentrations have been controlled for by glycogen depletion of all animals via overnight fasting before tissue collection. Although not directly measured in the present study, previous studies from our laboratory[3-5] and others[28] using male Fischer 344 rats of similar ages have shown resting plasma hormone levels to be unchanged with age. Starnes et al[28] also reported the secretory response of the pancreas (insulin and glucagon) to be maintained throughout life in the male Fischer 344 rat.

Interestingly, PEPCK mRNA was also found to be significantly altered in old compared with young animals. At 27 months, old trained and untrained animals exhibited a 30% and 45% decline in PEPCK message compared with 7-month-old young animals. An inverse relationship between F 2,6-P_2 and PEPCK mRNA was found to exist ($r = .82$; Fig 3). The strong correlation between PEPCK mRNA and F 2,6-P_2 suggests a coordination between signals that regulate the concentration of this metabolite and gene expression. Both processes are regulated by cyclic adenosine monophosphate (cAMP) and insulin. However, given that insulin acts to increase F 2,6-P_2 levels and since aging has been shown to reduce insulin sensitivity,[29] the changes observed with aging in F 2,6-P_2 content may involve the other primary regulator, cAMP. Changes in F 2,6-P_2 and PEPCK mRNA with age occurred regardless of training condition. Endurance training therefore does not appear to be a potent enough stimulator to counteract these deleterious alterations in gluconeogenic regulation observed with age.

The present study has contributed to a growing number of studies investigating alterations in critical regulators of gluconeogenesis with age and/or training. Lupa et al[30] examined changes in lactate dehydrogenase activity with age and training. Since age and/or training have been reported to affect the gluconeogenic rate when lactate was the precursor,[3,5,7] the enzyme responsible for conversion of lactate to pyruvate was of particular interest. However, endurance training decreased lactate dehydrogenase activity in young animals only. This suggests that lactate dehydrogenase cannot account for changes in gluconeogenesis reported with age or training. Additionally, Sumida et al[7] did not observe any training effect on F 1,6-P_2ase activity despite finding elevated hepatic gluconeogenic capacity. Thus, other mechanisms must be responsible for the training effects observed in gluconeogenesis.

Bypassing the energetically favored glycolytic enzyme pyruvate kinase requires PEPCK, found in both the cytosol and the mitochondria of mammalian hepatocytes. Species-specific differences have been reported in the location of PEPCK within the hepatocyte. In rats, PEPCK is located predominately in the cytosol, whereas human concentrations of PEPCK are equally distributed between the mitochondrial and cytosolic components.[31,32] This is important since only the cytosolic form is thought to be susceptible to alterations in activity.[31]

The present study found evidence of an age-related decline in cytosolic PEPCK activity among middle-aged and old animals regardless of training status. Compared with young animals, maximal PEPCK activity declined 28% and 14% in trained and untrained middle-aged animals, respectively. Hepatic PEPCK activity declined 83% and 62% in old trained and untrained animals compared with young counterparts. These results support previous research that has identified a training-insensitive age-related decline in cytosolic PEPCK activity.[3-5]

Because PEPCK has been hypothesized as the major regulator of gluconeogenesis, the mitochondrial activity of this enzyme was also examined. Mitochondrial proliferation is a documented effect of endurance training in skeletal muscle. Therefore, it seemed possible that hepatic mitochondrial PEPCK

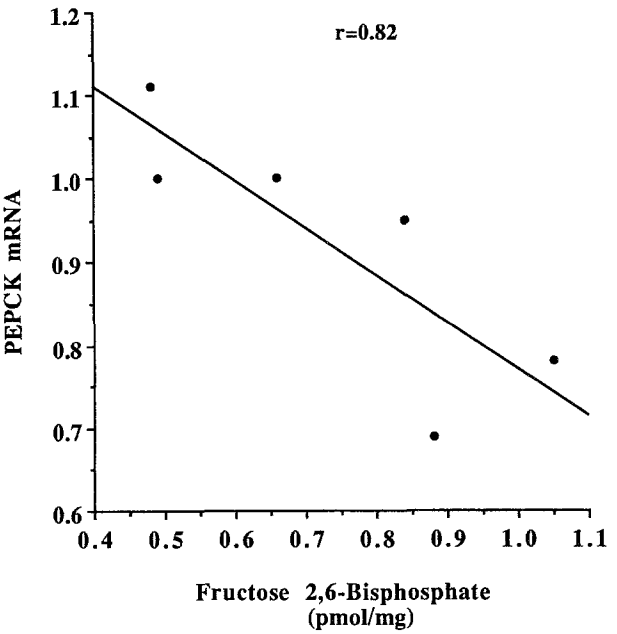

Fig 3. Relationship between hepatic F 2,6-P_2 levels and PEPCK mRNA for all 6 groups of animals examined.

could account for alterations previously documented in gluconeogenesis. The results of the present study found no significant differences between trained and untrained animals in mitochondrial PEPCK activity. This may be due to a lack of response to changes in liver mitochondrial content. Hepatic citrate synthase activity, also examined in these animals, was found to decline with age but was unaffected by training (Mazzeo et al, unpublished results, July 1996). Hepatic mitochondrial enzyme activity appears to be unaffected by training and differentially affected by age.

Pyruvate carboxylase is responsible for the conversion of pyruvate to oxaloacetate. In a review by Hers and Hue,[33] it is suggested that because gluconeogenic substrate concentrations (ie, pyruvate) are normally near the K_m of pyruvate carboxylase under physiological conditions, pyruvate carboxylase activity is largely dependent on substrate availability. In the present study, pyruvate carboxylase activity provided some insight into the regulation of gluconeogenesis with age, but not endurance training. Pyruvate carboxylase activity was reduced in old trained animals compared with young (42%) and middle-aged (37%) animals. Untrained old animals exhibited no such decline in activity compared with the younger counterparts. Since age-related declines have been found irrespective of training, the decreased activity of pyruvate carboxylase found in the old animals cannot explain the age-related declines in gluconeogenesis.

The present study found no changes in malate dehydrogenase activity as a function of age or training. However, this does not preclude involvement of related shuttle mechanisms. In an attempt to analyze the rate-limiting capability of cytoplasmic and mitochondrial shuttle systems, Berry and Kun[34] concluded that malate dehydrogenase and the malate-oxaloacetate shuttle are potentially rate-limiting. When malate dehydrogenase was inhibited using fluoromalate, a second shuttle system, the glutamate-aspartate shuttle, failed to compensate for the impaired malate-aspartate shuttle. Based on this, the investigators suggested that when gluconeogenesis is using predominately pyruvate, decreased malate dehydrogenase would impair gluconeogenic capability. However, the glutamate-aspartate shuttle predominates when lactate is the main precursor. Finally, they suggested that an alternative, as yet unidentified shuttle system probably exists as well.

In summary, alterations in F 2,6-P_2, cytosolic PEPCK activity, and PEPCK mRNA can account for some of the age-related decline previously reported in gluconeogenic capacity. It appears that declines in hepatic gluconeogenic capacity with age at the cellular level are widespread (ie, alterations in enzyme activity, gene expression, and metabolite concentration). The regulators examined in this study do not appear to play a role in the attenuation of the age-related decline previously found with endurance training. Examination of changes at the molecular level is warranted. Future research in this area might also focus on substrate availability and receptor sensitivity in response to both endurance training and aging.

REFERENCES

1. Bois-Joyeux B, Chanez M, Peret J: Age-dependent glycolysis and gluconeogenesis enzyme activities in starved-refed rats. Diabete Metab 16:504-512, 1990
2. Kmiec Z, Mysliwki A: Age-dependent changes of hormone-stimulated gluconeogenesis in isolated rat hepatocytes. Exp Gerontol 18:173-184, 1983
3. Podolin DA, Gleeson TT, Mazzeo RS: Role of norepinephrine in hepatic gluconeogenesis: Evidence of aging and training effects. Am J Physiol 267:E680-E686, 1994
4. Podolin DA, Pagliassotti MJ, Gleeson TT, et al: Influence of endurance training on the age-related decline in hepatic glyconeogenesis. Mech Ageing Dev 74:81-93, 1994
5. Podolin DA, Gleeson TT, Mazzeo RS: Hormonal regulation of hepatic gluconeogenesis: Influence of age and training. Am J Physiol 270:R365-R372, 1996
6. Exton JH, Park CR: Control of gluconeogenesis in liver. I. General features of gluconeogenesis in the perfused livers of rats. J Biol Chem 242:2622-2636, 1967
7. Sumida KD, Urdiales JH, Donovan CM: Enhanced gluconeogenesis from lactate in perfused livers after endurance training. J Appl Physiol 74:782-787, 1993
8. Wasserman DH, Cherrington AD: Hepatic fuel metabolism during muscular work: Role and regulation. Am J Physiol 260:E811-E824, 1991
9. Van Schaftingen E: Role of fructose-2,6-bisphosphate in the regulation of carbohydrate metabolism, in Pilkis SJ (ed): Fructose-2,6-Bisphosphate. Boca Raton, FL, CRC, 1990, pp 65-85
10. Donovan CM, Sumida KD: Training improves glucose homeostasis in rats during exercise via glucose production. Am J Physiol 258:R770-R776, 1990
11. Turcotte LP, Brooks GA: Effects of training on glucose metabolism of gluconeogenesis-inhibited short-term–fasted rats. J Appl Physiol 68:944-954, 1990
12. Winder WW, Holman RT, Garhart SJ: Effect of endurance training on liver cAMP response to prolonged submaximal exercise. Am J Physiol 240:R330-R334, 1981
13. Mazzeo RS, Brooks GA, Horvath SM: Effects of age on metabolic responses to endurance training in rats. J Appl Physiol 57:1369-1374, 1984
14. Srere PA: Citrate synthase. Methods Enzymol 13:3-5, 1969
15. Gohil K, Jones DA, Edwards RHT: Analysis of muscle mitochondrial function with techniques applicable to needle biopsy samples. Clin Physiol 1:195-207, 1981
16. Van Schaftingen E: D-Fructose-2,6-bisphosphate, in Bergmeyer HU (ed): Methods of Enzymatic Analysis. Weinheim, Germany, Verlag Chemie, 1984, pp 335-341
17. Petrescu I, Bojan O, Saied M, et al: Determination of PEPCK activity with deoxyguanosine 5′-diphosphate as nucleotide substrate. Anal Biochem 96:279-281, 1979
18. Friedman JE: Role of glucocorticoids in activation of hepatic PEPCK gene transcription during exercise. Am J Physiol 266:E560-E566, 1994
19. Minty AJ, Caravatti M, Robert B, et al: Mouse actin messenger RNAs. J Biol Chem 256:1008-1014, 1981
20. Crabtree B, Higgins SJ, Newsholme EA: The activities of pyruvate carboxylase, phosphoenolpyruvate carboxylase and fructose diphosphatase in muscles from vertebrates and invertebrates. Biochem J 130:391-396, 1972
21. Englard S, Siegel L: Mitochondrial L-malate dehydrogenase of beef heart. Methods Enzymol 8:98-108, 1969
22. Donovan CM, Brooks GA: Endurance training affects lactate clearance, not lactate production. Am J Physiol 244:E83-E92, 1983
23. Huston RL, Weiser PC, Dohm GL, et al: Effects of training, exercise and diet on muscle glycolysis and liver gluconeogenesis. Life Sci 17:369-376, 1975
24. Dohm GL, Kasperek GJ, Barakat HA: Time course changes in

gluconeogenic enzyme activities during exercise and recovery. Am J Physiol 249:E6-E11, 1985

25. Dohm GL, Newsholme EA: Metabolic control of hepatic gluconeogenesis during exercise. Biochem J 212:633-639, 1983

26. Pilkis SJ, El-Maghrabi MR, McGrane M, et al: Regulation by glucagon of hepatic pyruvate kinase, 6-phosphofructo 1-kinase, and fructose-1,6-bisphosphatase. Fed Proc Fed Am Soc Exp Biol 41:2623-2628, 1982

27. Pilkis SJ, El-Maghrabi MR, Pilkis J, et al: Fructose-2,6-bisphosphate: A new activator of phosphofructokinase. J Biol Chem 256:3171-3177, 1981

28. Starnes JW, Cheong E, Matschinsky FM: Hormone secretion by isolated perfused pancreas of aging Fischer 344 rats. Am J Physiol 260:E59-E66, 1991

29. Couet C, Delarue J, Constans T, et al: Age-related insulin resistance: A review. Horm Res 38:46-50, 1992

30. Lupa VA, Podolin DA, Roth DA, et al: Influence of aging and training on lactate dehydrogenase in liver and skeletal muscle. Mech Ageing Dev 75:191-204, 1994

31. Soling HD, Kleineke J: Species dependent regulation of hepatic gluconeogenesis in higher animals, in Hanson RW, Mehlman MA (eds): Gluconeogenesis: Its Regulation in Mammalian Species. New York, NY, Wiley, 1976, pp 369-462

32. Tilghman SM, Gunn JM, Fisher LM, et al: Reinduction of phosphoenolpyruvate carboxykinase (guanosine triphosphate) synthesis in Reuber H-35 cells. J Biol Chem 250:3322-3329, 1975

33. Hers HG, Hue L: Gluconeogenesis and related aspects of glycolysis. Annu Rev Biochem 52:617-653, 1983

34. Berry MN, Kun E: Rate-limiting steps of gluconeogenesis in liver cells as determined with the aid of fluoro-dicarboxylic acids. Eur J Biochem 27:395-400, 1972