Mechanisms of increased gluconeogenesis from alanine in rat isolated hepatocytes after endurance training

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Mechanisms of increased gluconeogenesis from alanine in rat isolated hepatocytes after endurance training. Am. J. Physiol. Endocrinol. Metab. 278: E35–E42, 2000.—This work aimed at further investigating the mechanisms by which liver gluconeogenic capacity from alanine is improved after training in rats, with an isolated hepatocyte model. Compared with controls in hepatocytes from trained rats incubated with gluconeogenic precursors (20 mM), the glucogenic flux (Jglucose) was increased by 64% from alanine (vs. 21% for glycerol, 18% for lactate-pyruvate-10.1, and 10% for dihydroxyacetone). Maximal intracellular alanine accumulation capacity was also increased by 50%. Further experiments conducted on perfused hepatocytes showed that the putative adaptation at the level of the phosphoenolpyruvate-pyruvate cycle, which could be involved in the increased Jglucose from lactate-pyruvate, was not involved in the increased Jglucose from alanine after training. For alanine concentration higher than ~1 mM, an increased flux through alanine aminotransferase appeared responsible for the increased Jglucose. This could, in turn, depend on an increased supply of cytosolic 2-oxoglutarate because of the higher mitochondrial respiration observed in hepatocytes from trained rats and the activation of the malate-aspartate shuttle. At lower alanine concentration, the increase in Jglucose appeared to be entirely due to the improved transport capacity.

Methods of increased gluconeogenesis from alanine, in isolated hepatocytes. In a first series of experiments, isolated hepatocytes from trained and control rats were incubated with saturating concentrations of various gluconeogenic precursors (lactate-pyruvate, glycerol, dihydroxyacetone, and alanine). Results from these experiments showed that training significantly increased maximal gluconeogenic flux from every substrate tested, particularly from alanine. Alanine transport capacity was then assessed in incubated hepatocytes, and the perfusion technique was used to identify the step(s) involved in the training activation of gluconeogenesis from alanine. Results from these experiments suggest that training-induced increase in gluconeogenesis from alanine is due to both increased transport and transamination into pyruvate.

Methods

Animal care and training program. The studies were conducted on male Wistar rats (initial weight, ~180 g). Animals were housed in pairs in a room with a 12:12-h light-dark cycle and were fed regular rodent lab chow with water ad libitum.

Trained animals swam in pairs in circular tanks (90 cm in diameter, filled to a depth of ~60 cm with water at 35°C agitated with compressed air) 5 days/wk for a period of 4 wk. Swimming duration was progressively increased from 30 min during the first week to 210 min during the fourth week. Data from Taguchi et al. (35) indicate that this swimming protocol results in significant training adaptations, as evidenced, for example, by a 25–30% increase in succinate dehydrogenase activity in the extensor digitorum longus and the deep portion of the vastus lateralis. Control animals were handled daily and were placed in water (35°C) for 10 min 5 days/wk.

Isolation of hepatocytes. Observations were performed 72 h after the last training session, after a 16-h fast. The rats were anesthetized with sodium pentobarbital (10 mg/100 g body wt), and the hepatocytes were isolated according to the method of Berry and Friend (5) as modified by Groen et al. (17). Briefly, the portal vein was cannulated, and a 2-min
PERFUSION OF HEPATOCYTES. Liver cells were perfused according to the method of van der Meer and Tager (37) modified by Groen et al. (17) as previously described (2). Hepatocytes (225–250 mg dry mass) were placed in 15-mL perfusion chambers at 37°C and were perifused (5 mL/min) with a 0.15, 0.30, 0.60, 1.2, 2.4, 4.8, and 9.6 mM lactate-pyruvate concentration was measured in the perfusate O2 content was monitored with Clark electrodes (Yellow Springs Instruments, Yellow Springs, OH) to assess O2 uptake of the hepatocyte suspension. After 40 min, when O2 uptake had reached a steady state, alanine was infused into the chamber at a constant rate to obtain several successive steady states with increasing alanine concentrations (0.15, 0.30, 0.60, 1.2, 2.4, 4.8, and 9.6 mM).

At each steady state, three samples (5 mL) of the perfusate were taken at 2-min intervals for subsequent determination of glucose, lactate, pyruvate, acetooacetate, and β-hydroxybutyrate concentrations. Samples were stored at 4°C and analyzed within 12 h after the end of the experiment. In addition, 300 µL of the cell suspension was sampled from the chamber for intra- and extracellular fractionation as described in Alainet transport in incubated hepatocytes.

Finally, another 300-µl sample was removed for cellular fractionation. For this purpose, mitochondrial and cytosolic spaces were separated with the digitonin fractionation method described by Zuurendonk and Tager (42). Briefly, the cell suspension was placed in a 22-mL Eppendorf tube in an isotonic medium containing 2 mL of digitonin (Merck, Lyon, France) at 4°C. After 15 s, the tube was centrifuged for 15 s at 10,000 g to precipitate mitochondria through the underlying 800-µl layer of silicon oil (Waker AR 20, Munich, Germany) into 250 µL HClO4 (10% mass/vol) + 25 mM EDTA. The supernatant (700 µL) was immediately removed, deproteinized with HClO4 (5% mass/vol), and neutralized.

Glucose 6-phosphate in intra- and extracellular fractions, intracellular alanine, and cytosolic phosphoenolpyruvate and 3-phosphoglycerate concentrations in neutralized acid-soluble cytosolic fraction were measured fluorimetrically (Iobin-Yvon) as described by Bergmeyer (4).

RESULTS

Glucoseogenesis in isolated incubated hepatocytes. When hepatocytes isolated from trained and control animals were incubated with no substrate or with various gluconeogenic precursors, a linear increase in glucose concentration was observed over time in the incubation medium, as shown in Fig. 1, left, for lactate-pyruvate and for alanine as examples. The glucose accumulation rate (glucose flux or J glucose) was significantly higher for hepatocytes from trained vs. control rats when no glucoseogenic precursor was added to the cell suspension (endogenous J glucose 0.93 ± 0.13 vs. 0.44 ± 0.06 µmol·g dry cells−1·min−1). Much higher J glucose (corrected for endogenous J glucose) was observed when glucoseogenic precursors were provided to the cells (Fig. 1, right), but the significant difference between trained and control groups remained. The largest differences were observed with alanine and glycerol (64 and 21%, respectively, vs. 18% with lactate-pyruvate and 10% with DHA).

When DHA was provided as a glucoseogenic precursor, lactate-pyruvate concentration was measured in the incubation medium. The concentration appeared larger with hepatocytes from trained vs. control rats (26.2 ± 4.6 vs. 20.8 ± 2.4 µmol/g dry cells after 45 min), although the difference did not reach statistical significance (not shown).

Effect of training on alanine transport. Figure 2, left, shows the accumulation of intracellular alanine over time when 2 mM alanine was added to the cell suspen-
E37

**INCREASED GLUCONEOGENESIS FROM ALANINE AFTER TRAINING**

E37

**Fig. 1.** Effect of training on gluconeogenesis from various precursors in isolated incubated hepatocytes. Left: glucose accumulation over 45-min incubation period in hepatocytes from control and trained rats in presence of saturating concentration of alanine (Ala; 20 mM) and lactate-pyruvate (La + Pyr.; 202 mM). Right: mean glucose flux computed from linear accumulation of glucose between 15 and 45 min during incubation with various precursors, or without substrates (no subst.). Results are means ± SE for n = 9 in each group. J glucose: gluconeogenic flux; DHA, dihydroxyacetone; Glyc, glycero.

**Fig. 2.** Effect of training on alanine transport capacity measured in incubated isolated hepatocytes in presence of aminooxyacetate (AOA). Left: intracellular alanine accumulation ([alanine]pyr) over 15-min incubation period. Right: Hanes plot of time [alanine]pyr against 1/time. Time to half-maximal accumulation (1/T) = x-intercept. Maximal accumulation = x-intercept/y-intercept. Results are means ± SE for n = 6 in each group. Statistical comparisons were made with Student’s t-test for unpaired samples: **P ≤ 0.005.

**Fig. 3.** The relationship found between cytosolic 3-phosphoglycerate (Fig. 5, top left), phosphoenolpyruvate (Fig. 5, top right), and pyruvate (Fig. 5, bottom left), on one hand, and J glucose on the other hand, were similar in hepatocytes from trained and control rats, i.e., a single relationship between J glucose and the concentration of the given intermediate was observed. In addition, over the range of concentrations of intermediates observed in the present experiment.

**Fig. 4.** Shows J glucose (flow rate in the chamber × glucose concentration in the perfusate) vs. alanine concentration in the medium during perfusion in hepatocytes from trained and control rats. As already observed in the incubation experiments, perfused hepatocytes from trained rats exhibited a higher maximal gluconeogenic flux compared with that of control rats: 1.80 ± 0.03 vs. 1.19 ± 0.06 µmol·g dry cells⁻¹·min⁻¹ at the highest alanine concentration (9.6 mM). The higher J glucose in hepatocytes from trained rats was not only observed at saturating alanine concentrations but also for subsaturating concentrations as low as 0.6 mM.

The relationships between the concentrations of selected intermediates, on one hand, and J glucose on the other hand, in hepatocytes isolated from trained and control rats, when perfused with increasing concentration of alanine, are shown in Figure 5. The relationship found between cytosolic 3-phosphoglycerate (Fig. 5, top left), phosphoenolpyruvate (Fig. 5, top right), and pyruvate (Fig. 5, bottom left), on one hand, and J glucose on the other hand, were similar in hepatocytes from trained and control rats, i.e., a single relationship between J glucose and the concentration of the given intermediate was observed. In addition, over the range of concentrations of intermediates observed in the present experiment.
In contrast, when $J_{glucose}$ was plotted against intracellular alanine concentration, the relationships were markedly different for hepatocytes isolated from trained and control rats and both reached a plateau (Fig. 5, bottom right). For intracellular alanine concentration higher than $1 \mu mol/g$ dry cells (corresponding to $1.2 \text{ mM}$ in the medium; Fig. 6, top), $J_{glucose}$ was markedly and significantly higher in hepatocytes from trained rats. Below this value, the two relationships could not be distinguished.

As shown on Fig. 6, top, intracellular alanine concentration increased linearly from $0.2$ to $6.5 \mu mol/g$ when alanine concentration in the medium was increased from 0 to $9.6 \text{ mM}$. Compared with hepatocytes from control rats, intracellular alanine concentration was slightly, but not significantly, lower in hepatocytes from trained rats. Figure 6, bottom, shows the relationship between pyruvate and intracellular alanine concentration. A saturation kinetic was observed in hepatocytes from both experimental groups. However, for a given intracellular alanine concentration, pyruvate concentration was higher in hepatocytes from trained compared with control rats.

Table 1 shows the ratio of NADH to NAD$^+$ in the cytosol and mitochondria computed, respectively, from the ratios of lactate to pyruvate and $\beta$-hydroxybutyrate to acetoacetate in the perifusate (40). These ratios were stable over time during the perifusion, and the data have been pooled. The ratios of NADH to NAD$^+$ were significantly lower in hepatocytes from trained vs. control rats, both in the cytosol and mitochondria. The ratio of mitochondrial to cytosolic NADH/NAD$^+$ was, however, two times higher in hepatocytes from trained vs. control rats.

**DISCUSSION**

Results from the incubation experiments with saturating substrate concentration show that training significantly increases gluconeogenic capacity from alanine (+64%), glycerol (+21%), lactate-pyruvate (+18%), and DHA (+10%). Apparently, no data are available in the literature concerning the effect of training on gluconeogenic flux from glycerol. In contrast, results obtained with alanine, DHA, and lactate-pyruvate are in accordance with previous reports on the effect of training on gluconeogenic capacity (31, 33, 34). Sumida and Donovan (33) found that in liver perfused in situ, glucose production from $[14C]$alanine in saturating concentration ($10 \text{ mM}$) was significantly increased by 28% after training. They also reported an ~12% increase in glucose production from $20 \text{ mM}$ DHA, although this result did not reach statistical significance. As for lactate-pyruvate, Sumida et al. (34) have reported that maximal glucose flux from $[14C]$lactate was $23\%$ higher in perfused liver from trained rats. The much larger increases reported by Podolin et al. (31) in liver slices from middle-aged and old rats incubated with $[14C]$lactate ($100$–$200\%$) could be due to the fact that the liver slices were incubated with glucagon and epinephrine. Indeed, training not only increases basal gluconeogenic flux but also its response to various hormonal factors such as norepinephrine (31) and glucagon (12, 13).

Lactate and alanine both enter the gluconeogenic pathway at the level of pyruvate, and Sumida and Donovan (33) observed a similar $23$–$28\%$ increase in $J_{glucose}$ from these two substrates after training. These authors, thus, hypothesized that these results could reflect a common training adaptation for both sub-
strates at the level of the phosphoenolpyruvate-pyruvate cycle, favoring pyruvate phosphorylation. However, they did not exclude the possibility that the results for lactate and alanine arose from specific adaptations for each substrate. Indeed, the observation made in the present study, that training increases the gluconeogenic capacity from various precursors that enter gluconeogenesis at very different levels, suggests that the adaptation of this pathway to exercise training probably does not occur at a single step but could be spread out at various levels in this pathway and could be, at least partly, different for each precursor. The purpose of the present study was to try to identify those adaptations responsible for the increased gluconeogenic flux from alanine. This increase was not only the largest observed in the incubation experiment at saturating alanine concentration, but it was also observed in the perfusion experiment from subsaturating alanine concentrations as low as 0.6 mM (Fig. 4), which is only slightly higher than the range of concentration observed at rest and during exercise (0.2–0.5 mM; Refs. 1, 16).

As suggested by Sumida and Donovan (33), the increase in \( J_{\text{glucose}} \) from lactate-pyruvate after training points to an adaptation at the level of the phosphoenolpyruvate-pyruvate cycle, which has a flux control coefficient for gluconeogenesis of \( \approx 60\% \) with lactate-pyruvate as precursors (18, 32). Accordingly, an increase in gluconeogenesis from lactate-pyruvate could be due to an increase in the activity of pyruvate carboxylase (PC) and/or of phosphoenolpyruvate-carboxykinase (PEPCK) or to a reduction in the activity of pyruvate kinase (PK). As for the effect of training on PK activity, no data appear to be available. Results from the present experiment with DHA as precursor, however, suggest that training does not reduce PK activity. Indeed, when DHA is provided to isolated hepatocytes, a portion enters the glycolytic pathway and the lactate-pyruvate flux reflects the activity of PK (3). In the present study, lactate-pyruvate concentrations in the medium were slightly, albeit not significantly, higher when hepatocytes from trained rats were incubated with DHA (26.2 ± 4.6 vs. 20.8 ± 2.4 \( \mu \text{mol/g dry cells} \) after 45 min of incubation). These figures underestimate the flux through PK because in incubation experiments a portion of the lactate-pyruvate produced is converted into glucose. This underestimation could be larger with hepatocytes from trained rats, because gluconeogenesis from lactate-pyruvate was increased after training. Taken together, these results therefore suggest that the putative training adaptation at the level of the phosphoenolpyruvate-pyruvate cycle is due to the activation of PC and/or PEPCK, rather than to an inhibition of PK.

The effect of training on PEPCK and PC has been assessed in several studies (19, 20, 34, 41). However, no change in the maximal activity of PEPCK (19, 20, 34, 41) and PC (19, 20) and in PEPCK mRNA content (19) was reported after training. Although the activity of...
Fig. 6. Effect of training on alanine transport and transamination in perfused hepatocytes. Relationships between intra- and extracellular alanine concentration (top) and between pyruvate and intracellular alanine concentration (bottom) during perfusion in hepatocytes from control and trained rats. Results are means ± SE for n = 5 in each group.

PEPCK is entirely controlled by the rate of transcription and the concentration of this enzyme (30), these findings remain compatible with the consistent observation of a higher gluconeogenic flux from lactate-pyruvate observed after training by Sumida et al. (34), and the concentration of this enzyme (30), these findings remain compatible with the consistent observation of a higher gluconeogenic flux from lactate-pyruvate observed after training by Sumida et al. (34), table 1. Mitochondrial and cytosolic NADH/NAD⁺ in hepatocytes from control and trained rats during perfusion with alanine and ratio between mitochondrial and cytosolic redox states.

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<tr>
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<th>Cytosol</th>
<th>Mitochondria</th>
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<tbody>
<tr>
<td>L/P</td>
<td>NADH/NAD⁺</td>
<td>β-OH/AA</td>
</tr>
<tr>
<td>Control</td>
<td>4.61±0.84</td>
<td>5.12±0.93</td>
</tr>
<tr>
<td>Trained</td>
<td>5.32±0.17*</td>
<td>1.68±0.22*</td>
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Values are means ± SE of pooled data (n = 40) in hepatocytes from control and trained rats. Cytosolic and mitochondrial NADH/NAD⁺, respectively, were computed from lactate-to-pyruvate (L/P) and β-hydroxybutyrate-to-acetoacetate (β-OH/AA) ratios, with equilibrium constant of lactate dehydrogenase (1.11 × 10⁻⁶) and β-hydroxybutyrate dehydrogenase (4.93 × 10⁻⁴) (36). Measurements were made at each steady state during perfusion and remained stable throughout the experiments. Mito/Cyto, ratio between mitochondrial and cytosolic redox states. Statistical comparisons were made with Student’s t-test for unpaired samples. *P < 0.05.

Sumida and Donovan (33), and Podolin et al. (31), as well as in the present experiment. Indeed, the gluconeogenic flux across the phosphoenolpyruvate-pyruvate cycle not only depends on the activity of the enzymes controlling this cycle but also on the actual concentration of the substrates for the various enzymes in the condition studied. In this respect, it could be hypothesized that training increases the supply of oxaloacetate to PEPCK, which, in turn, increases the gluconeogenic flux without any change in the concentration and/or activity of the enzymes involved in the phosphoenolpyruvate-pyruvate cycle. No data are currently available on the effect of training on oxaloacetate transport across the mitochondrial membrane, and/or its concentration in the cytosol when lactate-pyruvate is provided to the hepatocyte, to support this hypothesis. In contrast, as discussed below, results from the present experiment rule out this hypothesis when alanine is the gluconeogenic precursor.

In fact, results from the perifusion experiment, which closely mimics the behavior of the hepatocyte in vivo, indicate that putative adaptations at the level of the phosphoenolpyruvate-pyruvate cycle, and/or downstream of this cycle, are not involved in the increased gluconeogenic flux from alanine. This is indicated by the observation that the relationship between pyruvate concentration and J glucose was similar for hepatocytes from trained and control rats (Fig. 5, bottom left). This single relationship indicates that for a given pyruvate concentration, J glucose is not modified by training. The observed increase in J glucose from alanine is solely due to the increased pyruvate concentration and does not depend on possible adaptations at the level of the phosphoenolpyruvate-pyruvate cycle. A single relationship was also found between cytosolic phosphoenolpyruvate and 3-phosphoglycerate concentrations, on one hand, and J glucose on the other hand (Fig. 5, top). These observations further suggest that the increased J glucose from alanine after training is not due to adaptations at steps located downstream from pyruvate. Alternatively, it cannot be ruled out that training adaptations occur between pyruvate and glucose in the gluconeogenic and glycolytic pathways but that these adaptations cancel each other so that no net effect on the gluconeogenic flux is observed.

These findings are in agreement with several consistent data showing that alanine metabolism in the liver is controlled upstream of pyruvate, by its transport into the hepatocyte for low extracellular concentrations (<1 mM; Refs. 14, 17, 27) and by its transamination into glucose, on the other hand (Fig. 5, top). These observations further suggest that the increased J glucose from alanine after training is solely due to the increased pyruvate concentration and does not depend on possible adaptations at the level of the phosphoenolpyruvate-pyruvate cycle. A single relationship was also found between cytosolic phosphoenolpyruvate and 3-phosphoglycerate concentrations, on one hand, and J glucose on the other hand (Fig. 5, top). These observations further suggest that the increased J glucose from alanine after training is solely due to the increased pyruvate concentration and does not depend on possible adaptations at the level of the phosphoenolpyruvate-pyruvate cycle. 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It can thus be hypothesized that the training-induced increase in gluconeogenic flux observed in the present experiment at high alanine concentration (1.2–9.6 mM) is entirely due to an increased transamination, which has a high flux control coefficient for gluconeogenesis in these conditions (8, 26). Data from the perifusion experiment confirm that at high alanine concentration the transamination step controls the gluconeogenic flux from alanine. This is evidenced by the linear increase in intracellular alanine concentration, without any trend to plateau, over the entire range of extracellular alanine concentration (Fig. 6, top). In contrast, the relationships between intracellular alanine concentration and \( J_{\text{glucose}} \), both of which plateaud for alanine concentration in the medium higher than –4 mM, and the plateau reached were markedly higher in hepatocytes from trained vs. control rats (1.80 ± 0.03 vs. 1.19 ± 0.06 µmol·g dry cells \(^{-1} \)·min \(^{-1} \)). A similar observation was made for the relationships between intracellular alanine concentration and pyruvate (maximal pyruvate concentration: 18 vs. 9 µM in the perfusate from hepatocytes from control and trained rats), although the plateau was not reached in hepatocytes from trained rats (Fig. 6, bottom). Taken together, these observations confirm that at high alanine concentrations, transamination is the main step controlling the gluconeogenic flux from alanine and indicate that training increases this flux by increasing the capacity of the hepatocyte to convert alanine into pyruvate.

In contrast, the observed increase in \( J_{\text{glucose}} \) at low alanine concentration in the medium (0.6 and 1.2 mM) could be mainly due to an increased alanine transport capacity. Data from several in vitro experiments, indeed, indicate that alanine transport into the hepatocyte has a high flux control coefficient for gluconeogenesis in these conditions (14, 17, 27). Data from Wasserman et al. (38) also show that in response to exercise in dogs, the increase in alanine conversion into glucose parallels the increase in its uptake across the liver. In the present experiment, during perifusion at low alanine concentration (<1.2 mM), the relationship between intracellular alanine concentration (<0.8 µmol/g dry cells) and \( J_{\text{glucose}} \) in hepatocytes from trained rats cannot be distinguished from that in hepatocytes from control rats. This observation suggests that, in these conditions, the increased capacity for alanine transamination brought about by training is not responsible for the increase in \( J_{\text{glucose}} \). The larger gluconeogenic flux observed (Fig. 4) could rather be due to the marked increased in alanine transport capacity across the plasma membrane, which is clearly demonstrated in incubated hepatocytes in the presence of aminooxycetate as a transaminase inhibitor (Fig. 2).

The capacity of alanine aminotransferase (60 µmol·g dry wt \(^{-1} \)·min \(^{-1} \); Ref. 33) is much higher than that of alanine transport in the liver (20 µmol·g dry wt \(^{-1} \)·min \(^{-1} \); Ref. 23). In addition, data concerning the effect of training on alanine aminotransferase activity are both limited and conflicting (21, 33). Alanine transamination could, however, control gluconeogenesis if 2-oxoglutarate supply in the cytosol becomes insufficient (8). The supply of cytosolic 2-oxoglutarate depends on the activity of the malate-aspartate shuttle, which, in turn, depends on the rate of mitochondrial respiration (i.e., \( V_{O_2} \)) and on the inner mitochondrial membrane potential (\( \Delta \mu_{\text{ij}} \); Ref. 29). In the present experiment, \( V_{O_2} \) was consistently higher in hepatocytes from trained vs. control rats (Fig. 3). This observation was already made by Bobyleva-Guarriero and Lardy (7) and is consistent with the reported higher malate dehydrogenase activity reported by Ji et al. (21) in livers from trained rats. As for \( \Delta \mu_{\text{ij}} \), the ratio between the mitochondrial and cytosolic redox states [(NADH/NAD\(^{+}\))/(NAD/NAD\(^{+}\))] in the perifusion experiment also suggests that it was significantly higher in livers from trained rats. Indeed, the large difference between the redox states in the mitochondria and the cytosol depends on the activity of the glutamate-aspartate carrier of the malate-aspartate shuttle, which is driven by \( \Delta \mu_{\text{ij}} \) (24). In hepatocytes from trained rats, the segregation of reducing equivalents in the mitochondria vs. the cytosol was two times higher than in hepatocytes from control rats (Table 1), strongly indicating that \( \Delta \mu_{\text{ij}} \) was also higher. It is therefore tempting to speculate that the observed increase in alanine transamination into pyruvate could be due to an increased mitochondrial respiration in hepatocytes from trained vs. control rats. The associated stimulation of the malate-aspartate shuttle could, in turn, increase the supply of cytosolic 2-oxoglutarate, thus promoting alanine transamination into pyruvate and gluconeogenesis. In addition, the increase in mitochondrial respiration could also explain the increased \( J_{\text{glucose}} \) from glycerol observed in incubated hepatocytes from trained rats. It is, indeed, well established that an increased \( J_{\text{glucose}} \) from glycerol is entirely dependent on the rate of removal of cytosolic reducing equivalents (6, 25, 39).

In conclusion, results from the present experiment confirm that gluconeogenesis from alanine is increased in isolated hepatocytes from trained vs. control rats, both at low (near physiological) and high (saturating) concentrations. This increase is not due to adaptations at the level of the phosphoenolpyruvate-pyruvate cycle or at steps located downstream from this cycle. It is rather due to an increased alanine transport capacity at low alanine concentration and to an increased transamination of alanine into pyruvate at high alanine concentration. This later phenomenon could, in turn, be due to the increased mitochondrial respiration observed in hepatocytes from trained vs. control rats, which activates the malate-aspartate shuttle and increases the supply of cytosolic 2-oxoglutarate.
Increased gluconeogenesis from alanine after training


