Désy, François, Yan Burelle, Patrice Bélanger, Marielle Gascon-Barré, and Jean-Marc Lavioie. Effects of acute exercise on the gluconeogenic capacity of periporal and perivenous hepatocytes. *J Appl Physiol* 91: 1099–1104, 2001.—The present study was conducted to examine the effect of a single bout of exercise (rodent treadmill, 60 min at 26 m/min, 0% grade) on the gluconeogenic activity of periporal hepatocytes (PP-H) and perivenous hepatocytes (PV-H) in fasted (18 h) rats. Isolated PP-H and PV-H, obtained by selective destruction following liver perfusion with digitonin and collagenase, were incubated with saturating concentrations of alanine (Ala; 20 mM) or a mixture of lactate and pyruvate (Lac + Pyr; 20:2 mM) to determine the glucose production flux ($J_{\text{glucose}}$) in the incubation medium. Results show that, in the resting conditions, $J_{\text{glucose}}$ from all exogenous substrates was significantly higher ($P < 0.01$) in PP-H than in PV-H. Exercise, compared with rest, resulted in a higher $J_{\text{glucose}}$ ($P < 0.01$) from Lac + Pyr substrate in the PV-H but not in the PP-H, resulting in the disappearance of the difference in $J_{\text{glucose}}$ between PP-H and PV-H. Exercise, compared with rest, led to a higher $J_{\text{glucose}}$ ($P < 0.01$) from Ala substrate in both PP-H and PV-H. However, the exercise-induced increase in $J_{\text{glucose}}$ (gluconeogenic activity) from Ala substrate was higher in PV-H than in PP-H, resulting, as from Lac + Pyr substrate, in the disappearance ($P > 0.05$) of the difference of $J_{\text{glucose}}$ between PP-H and PV-H. It is concluded that exercise differentially stimulates the gluconeogenic activity of PV-H to a larger extent than PP-H, indicative of a heterogenous metabolic response of hepatocytes to exercise.

**METABOLIC ZONATION IS A 25-year-old concept proposed by Sasse et al. (30) on the basis that hepatocytes, depending on their position along the acinus, possess different amounts and activities of enzymes and thus different metabolic capacities. Several recent reviews have shown that hepatocytes also differ in a variety of morphometric, histochemical, and biochemical characteristics depending on their location (10, 13, 17–19). Following the bloodstream along the liver acinus, at least two different zones can be discerned. The first zone includes hepatocytes located in proximity to the terminal portal venules or periporal hepatocytes (PP-H), which are perfused with blood rich in O$_2$, substrates, and hormones. The second zone includes hepatocytes in the vicinity of terminal hepatic venules or perivenous hepatocytes (PV-H), which are perfused with blood containing lower concentrations of O$_2$, substrates, and hormones and with higher concentrations of CO$_2$ and other by-products of metabolism (17). Regarding carbohydrate metabolism, it appears that PP-H possess higher levels of gluconeogenic enzymes and PV-H possess higher levels of glycolytic enzymes (19). Thus periporal cells show a greater capacity for gluconeogenesis and perivenous cells for glycolysis even though all enzymes implicated in these metabolic pathways are present in both zones.

Supporting the view that metabolic zonation has a functional significance, it has been shown in studies, using anterograde and retrograde liver perfusions (4, 24, 25), that this heterogeneous expression of enzyme activity results in markedly different gluconeogenic and glycolytic capacities between the two populations of hepatocytes. More recently, direct measurements of glucose flux in isolated hepatocytes confirmed the zonation model for gluconeogenesis by demonstrating an up to twofold higher rate of glucose production from alanine (Ala) or a lactate and pyruvate mixture (Lac + Pyr) in PP-H compared with PV-H (6, 15).

An important characteristic of the zonation of liver carbohydrate metabolism is its dynamism and adaptability in response to many physiological and pathophysiological situations where the glucostat function of the liver is altered. These situations, in which metabolic zonation is modified to cope with the particular needs of the moment, include, among others, normal development (16), starvation (>24 h) (16, 21, 33), high-protein or high-fat diet (34), cold adaptation (31, 32), streptozotocin- and alloxan-induced diabetes (16, 27), thioacetamide-induced liver cirrhosis (28), and partial heptatectomy (16). Of these experiments, perhaps the strongest evidence of a metabolic zonation adaptation comes from Shiot et al. (31) who showed selectively higher glucose production from gluconeogenic precursors.
sors in PV-H but not in PP-H after 5 days of cold exposure.

In the latter experiment (31), cold exposure was used as a long-term gluconeogenic stimulus. A much more common and perhaps even more powerful stimulus for gluconeogenesis is physical activity. It is well known that prolonged exercise increases gluconeogenesis (35), which is of major importance because the inhibition of this metabolic process can alter glucose homeostasis and consequently the capacity of performing activity (14). Thus the question that arises is whether the acute stimulation of gluconeogenesis by exercise causes a perturbation of the metabolic zonation. In the affirmative, the metabolic zonation adaptability could represent an important and perhaps fundamental aspect of liver adaptation in response to exercise.

The present study was conducted to examine the effect of exercise on the gluconeogenic capacity of PP-H and PV-H isolated from the liver of rats subjected to a submaximal exercise bout of 60 min on a rodent treadmill. Because gluconeogenic rates are best measured in cells prepared from livers derived from rats fasted for 18–24 h (2) and because gluconeogenesis, in this condition, can be easily measured with unlabeled substrates (31), the effect of exercise on glucose production flux ($J_{\text{glucose}}$) in PP-H and PV-H was studied in 18-h fasted rats for which the pool of glycogen can be considered negligible. The main finding of the present work is that exercise affects the gluconeogenic capacity in such a way that the PV-H are able to increase their glucose production to the level of the PP-H. This results in the disappearance of the difference in the gluconeogenic capacity between PP-H and PV-H seen at rest.

**METHODS**

**Animal care.** All experiments described in this report were conducted according to the directives of the Canadian Council on Animal Care. Male Sprague-Dawley rats (Charles River Canada, St-Constant, Québec) weighing 280–300 g were housed in pairs and allowed pelleted rat chow and tap water ad libitum for 10–14 days. Lights were on from 0700 until 1900, and the room temperature was maintained at 20–23°C. Two days after their arrival, all rats were submitted once a day to a 5-day habituation running protocol on a motor-driven rodent treadmill starting at 15 m/min (0% grade) for 15 min on the first day and progressively increased to 26 m/min for 50 min (0% grade) on the fifth day.

**Groups and exercise protocol.** Food was removed from the cages of all rats by 1700 the day before experimentation (~18 h fasting). Because the pool of glycogen in the fasted group can be considered negligible, all of the glucose production in this group was assumed to be the result of gluconeogenesis. On the day of the experiment, rats were divided into a rest and an exercise group. The experiments were run between 0930 and 1100. The exercise test consisted of running on the treadmill for 60 min at 26 m/min (0% grade). At the end of the running or resting period, all rats were rapidly anesthetized with pentobarbital sodium (40 mg/kg ip). PP-H and PV-H were obtained from different rats in each condition.

**Isolation of hepatocytes.** Selective isolation of PP-H and PV-H was done according to the technique described by Gascon-Barré et al. (10) with minor modifications. Briefly, the portal vein and superior vena cava were cannulated with blunt needles (16 gauge) for selective anterograde and retrograde perfusion and digitonin injection. Both needles were positioned to rest at ~1 cm to the liver. The liver was perfused in open circuit in the anterograde direction at 37°C, pH 7.4, with saturated (95% O2-5% CO2) perfusion medium at a rate of 25 ml/min for 5 min using a peristaltic pump (Cole Parmer Instrument, Vernon Hills, IL). The perfusion medium consisted of (in mM) 118.9 NaCl, 4.76 KCl, 1.19 KH2PO4, 1.19 MgSO4 (7H2O), and 25 NaHCO3. For selective destruction of PP-H or PV-H, 7 mM digitonin (Boehringer Manheim), dissolved in a buffer containing (in mM) 137 NaCl, 4.7 KCl, 1.1 CaCl2, 0.65 MgSO4, and 10 HEPES at 90°C, was infused at 37°C in the portal vein in the anterograde direction (PP-H destruction) or in the cava catheter in the retrograde direction (PV-H destruction) at a rate of 10 ml/min using a microinfusion pump (Harvard Apparatus model 55-111). The infusion was maintained until the characteristic discoloration pattern was observed (23). In most of the preparations, this was achieved in <30 s, the procedure being commonly longer for PV-H destruction. Subsequently, the liver was perfused with the perfusion medium for 5 min at a rate of 40 ml/min in open circuit in the opposite direction of the one used for the digitonin infusion. After this, collagenase (CLS-2, 274 U/mg, Worthington Biochemical), 0.35 g% in perfusion medium supplemented with 2.4 mM CaCl2, was infused at a rate of 12 ml/min for 7–8 min to achieve liver digestion. Digestion was considered complete when gentle finger pressure on a liver lobe resulted in fracture of the liver surface (2). The liver was thereafter excised and transferred to a petri dish where it was gently disrupted with forceps. The cell solution was filtered through a nylon mesh and centrifuged twice for 2 min at 50 g, and the supernatant was discarded. Finally, cells were resuspended in a Falcon tube with ~15 ml of ice-cold perfusion medium supplemented with 2.4 mM CaCl2. The upper part of the tube was gassed for 1 min with 95% O2-5% CO2 and left on ice for 1 h before incubation.

**Incubations.** Hepatocytes were incubated at 10 mg/ml dry weight estimated from the weight of the wet-packed cells (dry weight = wet weight/3.8; Ref. 2) in closed vials saturated with 95% O2-5% CO2 in a final volume of 2.8 ml [in mM: 118.9 NaCl, 4.76 KCl, 1.19 KH2PO4, 1.19 MgSO4 (7H2O), 25 NaHCO3, 2.4 CaCl2, pH 7.4, 37°C]. The dry weight of each hepatocyte preparation was subsequently determined by allowing a precise amount (500 µl) of the hepatocyte solution to dry out for 2 days in a warm dry area. The dry weight values of each hepatocyte preparation were subsequently used in the calculations. When a gluconeogenic substrate was present, 2% bovine serum albumin (Boehringer Manheim) combined with 2 mM oleate (Sigma Chemical) was added. Cells were left for an equilibration period of 20 min in a shaking (2 Hz), thermostated (37°C) water bath. At time zero, either no substrate, 20 mM final concentration Ala (Boehringer Manheim), or 20:2 mM Lac+Pyr (Boehringer Manheim) was added. Cells were incubated for 45 min, and sampling was made every 15 min. Metabolic reactions were stopped by deproteinizing the sample (400 µl) in HClO4 (final concentration = 5% vol/vol) and vortexing. After centrifugation (2 min at 16,000 g), the supernatant was removed and neutralized with KOH and (2N)-3-(N-morpholino)propanesulfonic acid (0.3 M) for subsequent glucose determination (1). The glucose accumulation over time was used to compute $J_{\text{glucose}}$. For all incubations with Lac+Pyr or Ala, $J_{\text{glucose}}$ computed values were corrected for endogenous glucose pro-
duction to obtain $J_{\text{glucose}}$ produced from the addition of substrates.

**Cell viability and PP-H- and PV-H-enriched preparations.** The exclusion of trypan blue by intact cells is a commonly used method to distinguish between intact and damaged cells. The disadvantage of this procedure is the subjective error involved in microscopic counting of cells (22). The most sensitive method for determination of the viability of isolated liver cells is gluconeogenesis from Lac (22). At a given damage of the membrane, it allows us to estimate the extent of disturbances of coordinated cellular syntheses. However, the results are not readily available. Because, in the present experiment, glucose production from gluconeogenesis was measured, glucose production in the basal fasted state was used as the ultimate criteria of cell viability to which $J_{\text{glucose}}$ measurements obtained under similar conditions were compared. The test of exclusion of trypan blue was, however, run on each individual sample to obtain a rapid estimation of the cell viability and to avoid the use of an unacceptable (~80%) hepatocyte preparation. Glutamine synthetase activity (26) was determined in PP-H and PV-H in resting and exercising animals to ensure the adequacy of the cell-enriched preparations.

Statistical analyses were performed by a two-way nonrepeated-measures ANOVA. Comparisons of glutamine synthetase activities were done by using the Student’s t-test. Statistical significance was achieved at $P < 0.05$.

**RESULTS**

**Activity of the marker enzyme in PP-H and PV-H.** The activity of glutamine synthetase was significantly ($P < 0.01$) higher in PV-H than in PP-H both at rest and after the exercise bout (Table 1).

**Glucose concentrations in incubations.** Whether PP-H and PV-H were incubated without any exogenous substrate (endogenous glucose production), with Lac+Pyr, or with Ala, a linear increase in glucose concentration in the incubation medium was observed over time in all experimental conditions. An example of this relation is shown in Fig. 1 for the experiments performed in the rest condition. The linearity of the glucose accumulation over time was used to compute $J_{\text{glucose}}$ shown in Figs. 2–4.

**Endogenous glucose production.** As expected, endogenous glucose production was very low for all types of hepatocyte preparations (Fig. 2). Acute exercise led to a somewhat higher endogenous glucose production ($P < 0.05$) compared with rest in PP-H but not in PV-H, resulting in a small but significant ($P < 0.05$) difference between PP-H and PV-H in the exercise condition.

<table>
<thead>
<tr>
<th>Cell Preparations</th>
<th>PP-H</th>
<th>PP-V</th>
<th>PP-H/PV-H</th>
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</thead>
<tbody>
<tr>
<td>Rest</td>
<td>45 ± 12</td>
<td>723 ± 2*</td>
<td>0.06</td>
</tr>
<tr>
<td>Exercise</td>
<td>46 ± 9</td>
<td>655 ± 87*</td>
<td>0.07</td>
</tr>
</tbody>
</table>

Values are means ± SE for $n = 6$ in each group. PP-H, periportal hepatocytes; PV-H, perivenous hepatocytes. *Significantly different from PP-H, $P < 0.01$.
and PV-H, respectively, yielding a PP-H/PV-H of 1.64. Taken together, these comparisons of $J_{\text{glucose}}$ values and PP-H/PV-H obtained in the fast-rest conditions indicate that the cell viability of our preparations was very high. The activity of glutamine synthetase has been used as a stable marker of selective enrichment for PV-H isolated by digitonin-collagenase methods (11) because this enzyme is localized exclusively within a small population of parenchymal cells that surround the terminal hepatic venules. The large differences in glutamine synthetase activity found in the present cell preparations, whether at rest or after exercise, indicate that the PP-H- and PV-H-enriched preparations were not altered by the exercise stimulus and also give a good indication that the digitonin-collagenase technique used in the present experiment allowed us to obtain PP-H- and PV-H-enriched preparations.

Because glucose production from glycogenolysis can be considered negligible in the fasted state, $J_{\text{glucose}}$ measured at rest and after exercise in fasted rats was assumed to be solely the result of the gluconeogenic activity. The main finding of the present experiment was that an acute bout of exercise in fasted rats resulted in an unequal increase of $J_{\text{glucose}}$ in PP-H and PV-H. Exercise resulted in the stimulation of $J_{\text{glucose}}$ from Ala in both PP-H and PV-H. However, the magnitude of this stimulation appears to be greater in PV-H than in PP-H, resulting in the disappearance of the portovenous differences observed in the rested state. The same phenomenon was seen when Lac+Pyr was used as substrate with the exercise-induced activation of $J_{\text{glucose}}$ observed only in the PV-H. The proportionally greater stimulation of gluconeogenesis by exercise in PV-H compared with PP-H led to an attenuation and even disappearance of the initial por-

3.31 ± 0.29 μmol·min⁻¹·g dry cells⁻¹ in PP-H and PV-H, respectively). $J_{\text{glucose}}$ from Ala. Exercise resulted in significantly ($P < 0.01$) higher $J_{\text{glucose}}$ from Ala in both PP-H and PV-H (42% for PP-H and 94% for PV-H; Fig. 4). However, as observed with Lac+Pyr, exercise resulted in a nonsignificant difference in portovenous zonation of the gluconeogenic capacity from Ala (1.79 ± 0.09 vs. 1.49 ± 0.11 μmol·min⁻¹·g dry cells⁻¹ in PP-H and PV-H, respectively).

DISCUSSION

The digitonin-collagenase hepatocyte isolation procedure used in the present experiment resulted in PP-H and PV-H preparations that produced glucose in the same range as those previously reported in studies conducted under similar conditions (fast, rest; Refs. 6, 15, 31). Shiota et al. (31), using incubations of hepatocytes in the presence of saturating substrate concentrations, reported gluconeogenic fluxes from Lac+Pyr of ~3.7 and ~2.3 μmol·min⁻¹·g⁻¹ in PP-H and PV-H, respectively (PP-H/PV-H = 1.6). In the same study, gluconeogenic fluxes from Ala were ~2.1 and ~1.0 μmol·min⁻¹·g⁻¹ in PP-H and PV-H, respectively (PP-H/PV-H = 2.1). These values compare well with results of the present experiment obtained in incubated hepatocytes isolated from resting-fasted rats. Indeed, $J_{\text{glucose}}$ values from Lac+Pyr were 3.45 and 2.19 μmol·min⁻¹·g⁻¹ in PP-H and PV-H, respectively, yielding a PP-H/PV-H of 1.58. The same $J_{\text{glucose}}$ values from Ala were 1.26 and 0.77 μmol·min⁻¹·g⁻¹ in PP-H and PV-H, respectively.
tovenous expression of the zonation of gluconeogenic capacity from both Lac+Pyr and Ala. The disappearance of the metabolic zonation effect with strong gluconeogenic stimulus is also illustrated by the decreasing PP/PP of \( J_{\text{glucose}} \) (Table 2). These data show that because the gluconeogenic stimulus caused by exercise becomes more intense, there is a clear trend to homogeneity (PP-H/PP-V = 1.00) in the gluconeogenic activity of PP-H and PV-H. Overall, the present data indicate that exercise increases gluconeogenic activity in both PP-H and PV-H but that the increase in PV-H is predominant over PP-H with stronger gluconeogenic stimuli.

In the only other study reporting the effects of exercise on gluconeogenesis using the isolated hepatocyte model (from whole liver), the authors observed that only hepatocytes isolated from trained but not untrained rats showed an increase in gluconeogenic activity during exercise (3). The disparity of these results for the untrained rats and that of the present study might be due to the use of somewhat different gluconeogenic precursors or to the use of a somewhat milder exercise intensity. Perhaps if these authors (3) had examined the effects of exercise on gluconeogenic activity in PV-H and PP-H instead of in a mixture of hepatocytes, they might have found a different response for the two types of cells. This interpretation, based on the present findings of a heterogeneous increase in the gluconeogenic response of the hepatocytes to the exercise stimulus, raised the view that the different types of hepatocytes should be considered when interpreting the metabolic effects of exercise on the liver. For instance, it could be speculated that the enhanced hepatic gluconeogenic capacity shown to occur with training in in situ perfused liver preparation (5, 9) could be the result of a greater adaptation of PV-H than PP-H.

Without exogenous gluconeogenic substrates, \( J_{\text{glucose}} \) depends mainly on hepatic glycogenolysis, which in the present case is very low. No difference in endogenous \( J_{\text{glucose}} \) between PP-H and PV-H was found in the resting state (Fig. 2). The higher endogenous \( J_{\text{glucose}} \) values found in PP-H compared with PV-H following exercise may be explained by a somewhat larger contribution of gluconeogenesis from endogenous substrate since it is documented that substantial hepatic proteinolysis (up to 0.23 g/h) occurs during exercise (20). Therefore, one could speculate that protein degradation persisted after hepatocyte isolation, thus making some gluconeogenic precursors available.

The mechanisms underlying the metabolic zonation adaptations are far from being well understood. Numerous factors could potentially be responsible for the larger increase in gluconeogenic activity in PV-H than in PP-H following exercise in the fast state. The main evidence points to the phospho(\( \text{enolpyruvate-carboxykinase} \)) cycle. It has been reported that the phospho(\( \text{enolpyruvate-carboxykinase} \)) cycle has a flux control coefficient for gluconeogenesis of −60% with Lac+Pyr as precursors (12, 29). In their study using cold acclimatization as gluconeogenic stimulus, Shiota et al. (31) proposed that the selective increase of the gluconeogenic capacity of the PV-H could be due to the increase in the enzymatic activity of phospho(\( \text{enolpyruvate-carboxykinase} \)) (PEPCK). They observed a greater increase in PEPCK activity in PV-H than in PP-H. The same phenomenon could have occurred in the present study based on the following. PEPCK is distributed heterogeneously across the liver acinus, and long-term starvation (84 h) causes an increase in the overall (all hepatocytes from one acinus) PEPCK activity (33). In exercise conditions, PEPCK activity measured from liver biopsy has been shown to increase (7, 36) or remain constant (8) from rest to exercise. Therefore, it is possible that exercise could cause an increase in PEPCK activity, particularly in the PV-H, and that this would be responsible, at least in part, for the perturbation of the metabolic zonation observed in the present study.

The increase in \( J_{\text{glucose}} \) subsequent to an increase in PEPCK activity is, however, more likely to take place when Lac+Pyr, instead of Ala, is used as gluconeogenic precursor. At high concentrations of Ala, it appears that the increase in gluconeogenic flux is mostly due to an increased transamination (the other factor being membrane transport), which has a high flux control coefficient for gluconeogenesis in these conditions (5). It is well known that Ala aminotransferase is mostly located in the perportal zone (17). However, to our knowledge, the effect of exercise on Ala aminotransferase activity or its zonation is not known. Therefore, it could be hypothesized that the loss of hepatic zonation of gluconeogenesis from Ala in the fast-exercise condition could be because of a proportionally greater increase in flux through transamination in PV-H compared with PP-H.

In summary, taking into account that the present experiment was conducted on isolated hepatocytes using saturating concentrations of gluconeogenic precursors (Ala and Lac+Pyr) in the postexercise situation, the present results in fasted rats indicate that the PP-H have higher gluconeogenic capacities than PV-H in resting conditions. Exercise increased glucose production from Ala in both cell populations (PP-H and PV-H); however, when Lac+Pyr was used as gluconeogenic precursor, increased glucose production was observed in PV-H only. The PV-H were able to increase their glucose production following exercise from both precursors (Ala and Lac+Pyr) to the level of PP-H, resulting in the disappearance of the difference in the gluconeogenic contribution between PP-H and PV-H. The present data point to the recommendation that

Table 2. PP-H-to-PP-V ratios of \( J_{\text{glucose}} \) from Lac+Pyr or Ala at rest and after the exercise bout

<table>
<thead>
<tr>
<th></th>
<th>Lac+Pyr</th>
<th>Ala</th>
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<tbody>
<tr>
<td>Rest</td>
<td>1.58</td>
<td>1.64</td>
</tr>
<tr>
<td>Exercise</td>
<td>1.15</td>
<td>1.20</td>
</tr>
</tbody>
</table>

Ratios were computed from the mean glucose production flux (\( J_{\text{glucose}} \)) values of each group. Lac+Pyr, lactate + pyruvate; Ala, alanine.
hepatic zonation should be considered when analyzing the contribution of the liver to energy metabolism during exercise.

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REFERENCES