Metabolic Responses to Moderate Exercise in Lambs With Aortopulmonary Shunts

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In a previous study we found, after an overnight fast of 18 hours, a lower arterial glucose concentration and a depressed glycogenolysis in lambs with aortopulmonary left-to-right shunts. During exercise, glucose and free fatty acids (FFA) concentrations normally increase. The aim of this study was to investigate whether the shunt lambs could compensate for a depressed glycogenolysis by increasing gluconeogenesis and by increasing levels of blood substrates such as FFA and glycerol during exercise. Therefore, we investigated glucose kinetics, with [U-^{13}C]glucose, in five 7-week-old shunt and 7 control lambs of a similar age, at rest and during moderate exercise (treadmill; 50% of $\dot{V}O_2$ peak). The glucose production rate and the rate of disappearance of glucose were lower in shunt than in control lambs, both at rest and during exercise. We found no difference in metabolic clearance rate of glucose, glucose recycling, or gluconeogenesis between both groups of lambs. Glycogenolysis was at rest lower in shunt than in control lambs and tended to be lower during exercise. The arterial concentrations of pyruvate, lactate, FFA, and total and free glycerol increased during exercise in both groups of lambs. In conclusion, shunt lambs have lower arterial glucose concentrations than control lambs, both at rest and during moderate exercise. This was due to a lower glucose production rate, in particular a lower glycogenolysis. In addition, the reduced glycogenolysis rate was not offset by an increase in gluconeogenesis nor by an increase in other substrates that can be utilized by working muscles.

IN A PREVIOUS STUDY we found, at rest after an overnight fast of 18 hours, a lower arterial glucose concentration and a lower glucose production rate in lambs with an aortopulmonary left-to-right shunt.[1] Glucose can be produced by either gluconeogenesis or glycogenolysis. We found a decreased glycogenolysis in the shunt lambs and speculated that the glycogen stores were limited and readily depleted in the shunt lambs. During exercise there is an increasing demand for glucose by the working muscles, because they utilize more glucose during exercise than at rest.[2-8] Moreover, most studies have shown that during moderate exercise of limited duration, arterial concentrations of glucose and free fatty acids (FFA) increase.[9-11] Therefore, the glucose production or rate of appearance (R_a) of glucose need to be augmented, not only to meet the increased utilization or rate of disappearance (R_d) of glucose, but also to realize the expected increase in the arterial glucose concentrations. The increase in adrenaline and noradrenaline that occurs during exercise favors a rise in hepatic glucose production and favors glycogenolysis in muscle cells.[11,12]

We hypothesized that in shunt lambs the ability to increase the glucose production rate during exercise through an increase in glycogenolysis would be limited and we wondered whether the shunt lambs would compensate for this lack of glycogenolytic capacity by increasing gluconeogenesis and/or by increasing the plasma concentration of other energy substrates. Therefore, we measured the R_a and R_d of glucose, glycogenolysis, and gluconeogenesis with the aid of [U-^{13}C]glucose, at rest and during moderate exercise on a treadmill, in lambs with an aortopulmonary left-to-right shunt and in control lambs. In addition we determined the arterial concentrations of energy substrates, hemodynamic variables, and blood gas values. The plasma concentrations of adrenaline and noradrenaline were measured because of their important role in the regulation of energy metabolism during exercise.[11]

MATERIALS AND METHODS

We studied 12 seven-week-old lambs of mixed breed with documented dates of birth. They were randomly assigned to 2 groups: 5 lambs with an aortopulmonary shunt and 7 lambs without a shunt. Surgical preparation, catheter care, and antibiotic administration were performed as described previously.[13] In the shunt lambs, a Goretex conduit (inner diameter [ID] 6 mm; W.L. Gore, Flagstaff, AZ) was sutured between the descending aorta and the main pulmonary artery. Catheters were inserted into the aorta, the pulmonary artery, the right ventricle (only in the shunt lambs) and the right and left atria. Precalibrated electromagnetic flow transducers (ID 10 to 15 mm; Skalar Medical, Delft, The Netherlands) were placed around the ascending aorta just above the coronary arteries and around the pulmonary artery proximal to the conduit in the shunt lambs; in the control lambs a flow transducer was placed only around the pulmonary artery. Both the shunt and control lambs recovered quickly after surgery, and were returned to the stables to join their mothers on the day of the operation. Both groups of lambs were drinking breastmilk ad libitum. They were running and playing normally and did not show signs of being sick. There were no signs of infection or stress in either the shunt or control lambs. The experiments were performed with approval of the Committee on Animal Experiments of our university.

In the week before surgery and after recovery from surgery, the lambs were familiarized with running on a motor-driven treadmill (Laufergotest Junior, Erich-Jaeger, Hoechberg, Germany) during one short daily run. No training effect was pursued. The lambs ran freely on the treadmill without coercive measures. During the experiments an external work load corresponding to 50% of the peak oxygen consumption ($\dot{V}O_2$ peak) was used.

$\dot{V}O_2$ peak of each lamb was determined during a graded treadmill test 1 week after surgery as described previously.[14] After $\dot{V}O_2$ at rest had been calculated with the Fick formula (by measuring systemic blood-

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flow, and determining O_2 saturation and hemoglobin concentrations in aortic and mixed venous blood), the control lambs were subjected to a running speed of 3.5 km/h. After 3 minutes, $\dot{V}O_2$ was calculated again. Immediately after the blood samples had been collected, the work load was increased by setting the inclination at 4%. After another 3 minutes, the measurements were repeated, and so on, until the maximum inclination of 15% was reached. Thereafter, the treadmill speed was increased in steps of 0.5 km/h, while maintaining an inclination of 15%. The test was ended when a further increase in workload was no longer followed by a rise in $\dot{V}O_2$, ie, a plateau had been reached, or when the lamb showed clear signs of exhaustion. The criteria for exhaustion were swaying, open-mouthed panting, or imminent collapse. The graded treadmill test in the shunt lambs was modified by starting at a running speed of 2.5 km/h instead of 3.5 km/h. This modification was necessary because otherwise the speed and inclination corresponding to 50% of $\dot{V}O_2$ peak could not be determined.

Experimental Protocol

Between the 10th and 14th days after surgery the lambs were brought to the experimental room, after an overnight fast of 18 hours, weighed, and put on the treadmill. After 2 hours of habituation, when the lambs stood quietly, the first measurements were performed and blood samples were withdrawn. Systemic and pulmonary blood flow and aortic, pulmonary arterial and left atrial pressures were measured every 10 minutes for 1 hour.

To determine the glucose production rate and glucose recycling, [U-^{13}C]glucose was administered according to the prime dose constant-rate infusion technique.[15] Before starting the infusion of [U-^{13}C]glucose, blood samples were withdrawn from the aorta for determination of substrate concentrations (glucose, pyruvate, lactate, β-hydroxybutyrate, acetoacetate, FFA, total and free glycerol), adrenaline and noradrenaline concentrations, and the isotope ratio (^{13}C/^{12}C) of glucose to determine the natural abundance of ^{13}C in glucose. A priming dose of 7.3 mg $\cdot$ kg^{-1} [U-^{13}C]glucose (99 atom% ^{13}C; Isotec, Miamisburg, OH) was administered over 10 minutes into the right atrial catheter, followed by a constant-rate infusion (model 2620; Harvard Pump, Millis, MA) of 0.073 mg $\cdot$ min^{-1} $\cdot$ kg^{-1} [U-^{13}C]glucose.[16] During a steady state, 2 blood samples (at 30 and 40 minutes after starting infusion of the priming dose) were obtained from the aorta for determination of the substrate concentrations and the isotope ratio of glucose. At the same timepoints, blood samples were withdrawn with a heparinized syringe from the aortic and mixed-venous catheters, ie, from the right ventricular catheter in shunt lambs and the pulmonary arterial catheter in control lambs. Oxygen saturation was determined in all samples; hemoglobin concentration, pH, P_{CO_2}, P_{O_2}, and plasma HCO_3^-concentration were determined in the aortic sample.

Ten minutes later, speed and inclination of the treadmill were set to values that would impose a work load corresponding to approximately 50% of $\dot{V}O_2$ peak. The lambs had to run at this load for 30 minutes. At 10-minute intervals, blood flows and pressures were measured and blood samples were withdrawn for determination of oxygen saturation, hemoglobin, pH, P_{CO_2}, P_{O_2}, and plasma HCO_3^- substrate concentrations, and isotope ratio of glucose as described for the resting period. After the last blood sample had been withdrawn, the treadmill was stopped and the lamb was allowed to recover.

Measurements and Calculations

The precalibrated electromagnetic flow transducers were connected to Skalar MDL 400 flowmeters. Systemic and pulmonary blood flow rates were obtained in the shunt lambs from the pulmonary and the aortic flow transducers, respectively; systemic blood flow rate in the control lambs was obtained from the pulmonary flow transducer. Heart rate was obtained from the blood flow signal. Aortic, pulmonary arterial, and left atrial blood pressures were measured with Gould P23 ID pressure transducers (Spectramed, Oxnard, CA), referenced to atmospheric pressure with zero obtained with the pressure transducer at the right atrial level.[13] All variables were recorded on an Elema Mingograf 800 ink-jet recorder (Siemens-Elema, Solna, Sweden).

Oxygen saturation was determined with an OSM2 hemoximeter (Radiometer, Copenhagen, Denmark). Hemoglobin concentration was determined with the Haemocue method (B Hemoglobin Photometer, Haemocue, Helsingborg, Sweden). pH, P_{CO_2}, P_{O_2}, and plasma HCO_3^- concentration were determined with an ABL-2 blood gas analyzer (Radiometer).

Immediately after withdrawal, the blood samples were mixed with sodium fluoride to stop glycolysis, and kept in ice. Concentrations of glucose, pyruvate, lactate, β-hydroxybutyrate, acetoacetate, FFA, total glycerol, and free glycerol were determined by enzymatic methods.[17,18] Plasma adrenaline and noradrenaline concentrations were determined by high-pressure liquid chromatography (HPLC) with electrochemical detection.[19] After sample collection, the blood was centrifuged at 4°C. The thrombocyte-poor plasma was fortified with the antioxidant glutathione and stored at −20°C pending determination.

For the determination of the isotope ratio of glucose, the plasma was deproteinized with ethanol for 30 minutes at 4°C. After centrifugation, the supernatant was removed and dried under N_2. Pyridine and acetic anhydride (1:2 vol/vol) were added. This mixture was allowed to react for at least 24 hours at room temperature to form the penta-acetate derivative.[16] The samples were dried under N_2 and dissolved in 100 μL of hexane. The isotope ratio was determined by gas chromatography–mass spectrometry. A Hewlett-Packard (Hewlett-Packard, Palo Alto, CA) Model 5890 gas chromatograph was interfaced to a VG Trio-2 quadrupole mass spectrometer (Fisons Instruments, Manchester, UK). The mass spectrometer was used in the chemical ionization mode. Single ion monitoring was performed at mass-to-charge ratio (*m/e*) 408, 409, 410, 411, and 414, corresponding to $m + 0$, $m + 1$, $m + 2$, $m + 3$, and $m + 6$. Standards containing 0.0%, 1.5%, 3.0%, and 4.5% D-[U-^{13}C]glucose were prepared by diluting natural D-glucose with D-[U-^{13}C]glucose to obtain a calibration graph of isotope ratio versus molar fraction ($r = .998$; slope = 1.031). The molar fraction (F) of [U-^{13}C]glucose in the blood samples was calculated from this calibration graph.

Left-to-right shunt flow was obtained by subtracting systemic from pulmonary blood flow. Left-to-right shunt fraction was calculated by dividing left-to-right shunt flow by pulmonary blood flow. Blood oxygen concentration was calculated as the product of oxygen saturation, hemoglobin concentration, and a hemoglobin oxygen binding capacity of 1.36 mL/g.[20] Systemic oxygen supply was calculated as the product of arterial oxygen concentration and systemic blood flow. Whole body oxygen consumption ($\dot{V}O_2$) was calculated by multiplying the arterio-mixed venous oxygen concentration difference by systemic blood flow.

At rest, the glucose production rate (R_a, μmol $\cdot$ min^{-1} $\cdot$ kg^{-1}) was calculated as

$$R_a = \left(\frac{F_i}{F_{AO}} - 1\right) \cdot I \qquad (1)$$

where F_i is the molar fraction of [U-^{13}C]glucose in the infusate, F_{AO} the molar fraction of [U-^{13}C]glucose in the aorta at steady state and I is the rate of tracer infusion (μmol $\cdot$ min^{-1} $\cdot$ kg^{-1}). At rest, the glucose disappearance rate (R_d) equals R_a.

The gluconeogenic pathway through pyruvate and lactate (Cori cycle) was calculated from the fractional glucose ^{13}C recycling[21]

$$\text{Fractional glucose } ^{13}\text{C recycling} = \frac{3 \cdot F_{13C_3} + 2 \cdot F_{13C_2} + F_{13C_1}}{6 \cdot F_{13C_6} + 3 \cdot F_{13C_3} + 2 \cdot F_{13C_2} + F_{13C_1}} \qquad (2)$$

Gluconeogenesis via the Cori cycle was then calculated as the product of fractional glucose ^{13}C recycling and R_a. The sum of glycogenolysis and gluconeogenesis from other precursors such as glycerol and amino acids was estimated by subtracting the gluconeogenesis via the Cori cycle from R_a.

During exercise, R_a was calculated with a non–steady-state equation according to Steele[22]

$$R_a = \frac{I \cdot F_i - V \cdot \overline{C_{AO}} \cdot \frac{\Delta F_{AO}}{\Delta t}}{\overline{F_{AO}}} \quad (3)$$

where V is the average extracellular volume,[23] which was 281 and 312 mL/kg for control and shunt lambs, respectively.[24] $\overline{C_{AO}}$ is the mean concentration of glucose (μmol/mL) of the consecutive aortic samples, ΔF_{AO} is the difference in molar fraction of [U-^{13}C]glucose of the consecutive aortic samples, Δt is the time in minutes between the 2 samples, and $\overline{F_{AO}}$ is the mean molar fraction of [U-^{13}C]glucose of the consecutive aortic samples.

R_d then follows from

$$R_d = R_a - V \cdot \frac{\Delta C_{AO}}{\Delta t} \quad (4)$$

where ΔC_{AO} is the difference in concentration of glucose between the consecutive aortic samples. The metabolic clearance rate (MCR; mL $\cdot$ min^{-1} $\cdot$ kg^{-1}) was calculated by dividing R_d by the blood glucose concentration.

Statistical Analysis

Data are expressed as means ± SEM. To compare the hemodynamic variables and substrate concentrations between shunt and control lambs at rest, Student's 2-tailed *t* test for unpaired samples was used. To compare the hemodynamic variables, substrate concentrations, and glucose-related variables at rest with those at the various time periods during exercise, repeated-measures ANOVA was used. This was followed by Student's 2-tailed *t* test for paired samples. To compare the plasma concentrations of adrenaline and noradrenaline at rest between shunt and control lambs, a Wilcoxon signed rank test was used. To compare the plasma concentrations of adrenaline and noradrenaline at rest with those at the various time periods during exercise, a Wilcoxon signed rank test for matched pairs was performed. Linear regression analysis was performed using a statistical computer program (NCSS, Kaysville, UT). A P value $\leq .05$ was considered statistically significant.

RESULTS

Hemodynamic Data

$\dot{V}O_2$ peak experiment. The maximal absolute external work load achieved by the shunt lambs was significantly lower than that of control lambs (speed 3.5 ± 0.0 *v* 3.8 ± 0.1 km/h; $P < .001$; inclination 12% ± 1% *v* 15% ± 0%; $P < .01$). Hemodynamic data, oxygen-related variables, and plasma catecholamine concentrations at rest and during maximal exercise ($\dot{V}O_2$ peak) are shown in Table 1. $\dot{V}O_2$ at rest and $\dot{V}O_2$ peak were similar in both groups of lambs.

Moderate exercise. On the day of the study there were no statistically significant differences in age (44 ± 1 *v* 48 ± 3 days) and weight (13.4 ± 0.6 *v* 12.7 ± 0.5 kg) between the control and the shunt lambs. The absolute external work load, however, was lower in shunt than in control lambs (speed 2.9 ± 0.2 *v* 3.4 ± 0.1 km/h; $P < .05$; inclination 0.0% ± 0.0% *v* 0.6% ± 0.6%; $P < .05$). The mean left-to-right shunt fraction in the shunt lambs was at rest 49% ± 4% of pulmonary blood flow. The left-to-right shunt led to statistically significant differences in hemodynamic and oxygen-related variables between shunt and control lambs (Table 2). The hemodynamic responses to exercise was similar in shunt and control lambs, and most of the differences between the 2 groups at rest persisted during exercise. Systemic blood flow, systemic oxygen supply and $\dot{V}O_2$ were lower in shunt than in control lambs, both at rest and during moderate exercise.

Substrates

Moderate exercise. The shunt lambs had at rest and during exercise lower arterial concentrations of glucose than control lambs (Fig 1). During exercise, the arterial glucose concentra-

Table 1. Hemodynamic Data, Oxygen-Related Variables, and Plasma Catecholamine Concentrations at Rest and During Maximal Exercise ($\dot{V}O_2$ peak)

Variable	Rest		Exercise	
	Control	Shunt	Control	Shunt
Heart rate (beats/min)	138 ± 5	163 ± 11*	281 ± 13†	255 ± 11†
Mean pressures (mm Hg)				
Aortic	77 ± 2	59 ± 4*	78 ± 2	64 ± 4*
Pulmonary arterial	12 ± 3	16 ± 3	10 ± 2	18 ± 3*
Left atrial	4 ± 1	9 ± 4	2 ± 1	8 ± 4
Systemic blood flow (mL $\cdot$ min^{-1} $\cdot$ kg^{-1})	155 ± 2	146 ± 10	314 ± 10†	301 ± 30†
Effective stroke volume, left ventricle (mL/kg)	1.14 ± 0.04	0.91 ± 0.07*	1.13 ± 0.04	1.17 ± 0.07†
Oxygen saturation (%)				
Aortic	93 ± 1	93 ± 1	95 ± 1	94 ± 1
Mixed venous	50 ± 1	50 ± 4	26 ± 2†	28 ± 5†
Systemic oxygen supply (μmol $\cdot$ min^{-1} $\cdot$ kg^{-1})	725 ± 35	689 ± 46	1627 ± 50†	1545 ± 143†
$\dot{V}O_2$ (μmol $\cdot$ min^{-1} $\cdot$ kg^{-1})	343 ± 20	320 ± 32	1197 ± 54†	1194 ± 103†
Adrenaline (nmol/L)	0.8 ± 0.2	0.9 ± 0.5	6.6 ± 2.3†	6.2 ± 2.9
Noradrenaline (nmol/L)	4.1 ± 2.1	12.9 ± 6.5	11.5 ± 4.4	14.8 ± 6.5

NOTE. Data are means ± SEM; n = 7 control and 5 shunt lambs.

*$P < .05$, shunt *v* control.

†$P < .05$, rest *v* exercise.

Table 2. Hemodynamic Data and Oxygen-Related Variables at Rest and During Moderate Exercise (50% of $\dot{V}O_2$ peak)

Variable		Rest	Exercise 10 min	Exercise 20 min	Exercise 30 min
Heart rate (beats/min)	C	145 ± 10	218 ± 10†	232 ± 6†	240 ± 6†
	SH	135 ± 11	174 ± 15*†	189 ± 12*†	203 ± 17*†
Mean pressures (mm Hg)					
Aortic	C	96 ± 4	100 ± 2	99 ± 3	97 ± 4
	SH	77 ± 5*	78 ± 7*	83 ± 8*	83 ± 7*†
Pulmonary arterial	C	15 ± 2	20 ± 3†	22 ± 4†	20 ± 3†
	SH	22 ± 4	22 ± 6	24 ± 5	26 ± 5
Left atrial	C	3 ± 1	4 ± 1	2 ± 1	4 ± 3
	SH	17 ± 4*	18 ± 6*	19 ± 7*	17 ± 4*
Blood flow ($mL \cdot min^{-1} \cdot kg^{-1}$)					
Systemic	C	145 ± 6	215 ± 8†	231 ± 8†	235 ± 6†
	SH	119 ± 8*	172 ± 16*†	187 ± 17*†	193 ± 21*†
Pulmonary	C	145 ± 6	215 ± 8†	231 ± 8†	235 ± 6†
	SH	238 ± 12*	325 ± 27*†	335 ± 18*†	367 ± 21*†
O_2 saturation (%)					
Aortic	C	94 ± 1	95 ± 1†	95 ± 1†	95 ± 1†
	SH	94 ± 1	94 ± 1	93 ± 1	94 ± 1
Mixed venous	C	51 ± 3	38 ± 2†	39 ± 1†	40 ± 2†
	SH	51 ± 4	40 ± 4†	38 ± 4†	36 ± 2†
Hemoglobin (g/L)	C	82 ± 3	84 ± 2	83 ± 1	81 ± 2
	SH	78 ± 6	79 ± 7	75 ± 6	75 ± 7
Systemic O_2 supply	C	670 ± 49	1028 ± 48†	1101 ± 43†	1090 ± 43†
($\mu mol \cdot min^{-1} \cdot kg^{-1}$)	SH	521 ± 39*	772 ± 107*†	792 ± 115*†	827 ± 149
$^{\circ}O_2$ ($\mu mol \cdot min^{-1} \cdot kg^{-1}$)	C	279 ± 19	617 ± 22†	653 ± 26†	636 ± 36†
	SH	230 ± 12*	385 ± 38*†	399 ± 41*†	422 ± 39*†
Arterial					
PH	C	7.37 ± 0.02	7.38 ± 0.01	7.38 ± 0.01	7.38 ± 0.01
	SH	7.37 ± 0.02	7.38 ± 0.03	7.34 ± 0.02	7.39 ± 0.02
P_{CO_2} (Torr)	C	4.7 ± 0.1	4.4 ± 0.2†	4.4 ± 0.2†	4.3 ± 0.2†
	SH	5.2 ± 0.1*	5.0 ± 0.1*	5.2 ± 0.2*	4.9 ± 0.1*
P_{O_2} (Torr)	C	15.0 ± 0.7	14.9 ± 0.6	15.2 ± 0.7	15.3 ± 0.6
	SH	13.9 ± 0.6	14.6 ± 0.8	13.0 ± 0.5	13.7 ± 0.8
HCO_3^- (mmol/L)	C	19.5 ± 0.8	18.4 ± 0.8†	18.5 ± 1.0	18.2 ± 1.0†
	SH	21.5 ± 0.7	20.9 ± 0.9	20.0 ± 0.3	21.2 ± 0.8

NOTE. Data are means ± SEM; n = 7 control (C) and 5 shunt (SH) lambs.
*$P < .05$, shunt *v* control.
†$P < .05$, rest *v* exercise.

tion in control lambs rose gradually, whereas in the shunt lambs no increase in arterial glucose concentration was seen. Arterial concentrations of β-hydroxybutyrate tended to be lower in shunt than in control lambs, both at rest and during exercise. The arterial concentrations of pyruvate, lactate, FFA, and total and free glycerol increased during exercise in both groups of lambs. In the control lambs adrenaline and noradrenaline concentrations increased during exercise in comparison with at rest (Fig 2). In the shunt lambs only an increase in adrenaline and not in noradrenaline concentration occurred during exercise in comparison to at rest. However, there was no statistically significant difference in adrenaline and noradrenaline concentrations between shunt and control lambs.

Glucose Production, Gluconeogenesis, and Glycogenolysis

The R_a and R_d of glucose were lower in shunt than in control lambs, both at rest and during exercise (Fig 3), except at the timepoint of 10 minutes of exercise. In the control lambs, $R_a - R_d$ was considerably higher than in the shunt lambs, which is consistent with an arterial glucose concentration that rises during exercise in the control lambs (Fig 1). In the shunt lambs, $R_a - R_d$ was very low with as a consequence no increase in arterial glucose concentration during exercise. We found no difference in metabolic clearance rate of glucose, glucose recycling, or gluconeogenesis between the 2 groups of lambs (Fig 3). Glycogenolysis (which is the sum of glycogenolysis per se and gluconeogenesis from other precursors than glucose) at rest was lower in shunt than in control lambs and tended to remain so during exercise (Fig 3).

We found a positive correlation between R_a and $\dot{V}O_2$, and between R_a and the adrenaline concentration (Fig 4). There was no correlation between R_a and the noradrenaline concentration. R_a was also positively correlated to the arterial concentration of glucose ($r = .65$; $P = .0000$), lactate ($r = .56$; $P = .001$), FFA ($r = .33$; $P < .05$), total glycerol ($r = .46$; $P < .005$), and free glycerol ($r = .42$; $P < .01$). The metabolic clearance rate of

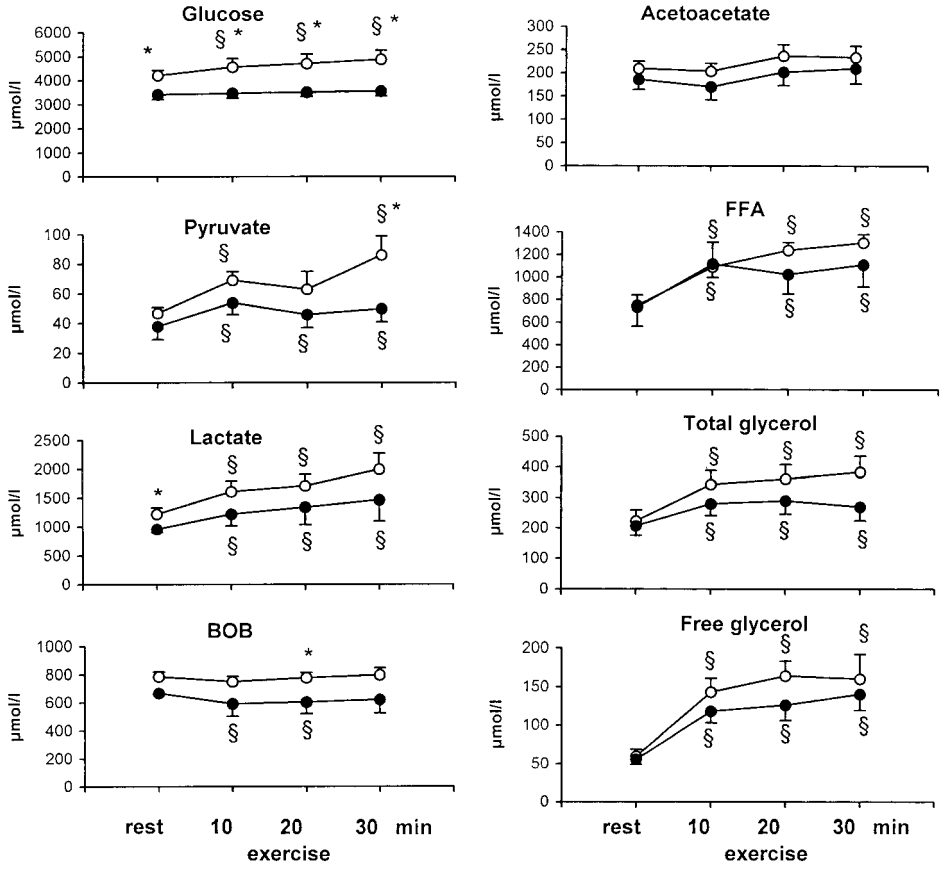

Fig 1. Arterial substrate concentrations of control (○; n = 7) and shunt (●; n = 5) lambs at rest and during moderate exercise (50% of $\dot{V}o_2$ peak). Data are means ± SEM. *$P < .05$: control *v* shunt. §$P < .05$: rest *v* exercise. BOB, β-hydroxybutyrate.

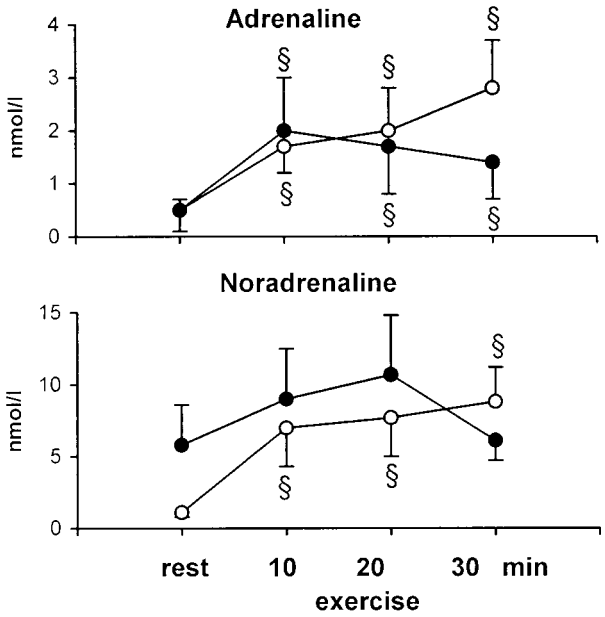

Fig 2. Adrenaline and noradrenaline concentrations of control (○; n = 7) and shunt (●; n = 5) lambs at rest and during moderate exercise (50% of $\dot{V}o_2$ peak). Data are means ± SEM. §$P < .05$: rest *v* exercise.

glucose was inversely related to the arterial concentration of FFA, total and free glycerol (Fig 5).

DISCUSSION

We found that, after an 18-hour fast, the arterial glucose concentrations were lower in shunt than in control lambs, both at rest and during exercise. During exercise, the arterial glucose concentrations rose gradually in control lambs, whereas in the shunt lambs the arterial glucose concentrations did not change. In the control lambs R_a exceeded R_d, whereas in the shunt lambs R_a and R_d were almost equal. The lack of increase in arterial glucose concentration during exercise in the shunt lambs was found to be due to a diminished R_a, in particular a diminished glycogenolysis (which is the sum of glycogenolysis per se and gluconeogenesis from other precursors than glucose). In the control lambs, there also was a temporary fall in R_d during the first 10 minutes of exercise, which suggests that the muscles initially utilized endogenous glycogen instead of exogenous glucose as a source of energy.

As demonstrated by others[25-28] in humans and in dogs, R_a increased during exercise in the control lambs. Furthermore, R_d increased consistent with a higher uptake of glucose by the muscles.[3-5,8,12] While glucose utilization increased in the control lambs, a rise in their arterial glucose concentration occurred as well, and was due to an increase in glycogenolysis. The shunt lambs did not increase the glucose production rate and maintained glucose homeostasis by not increasing glucose uti-

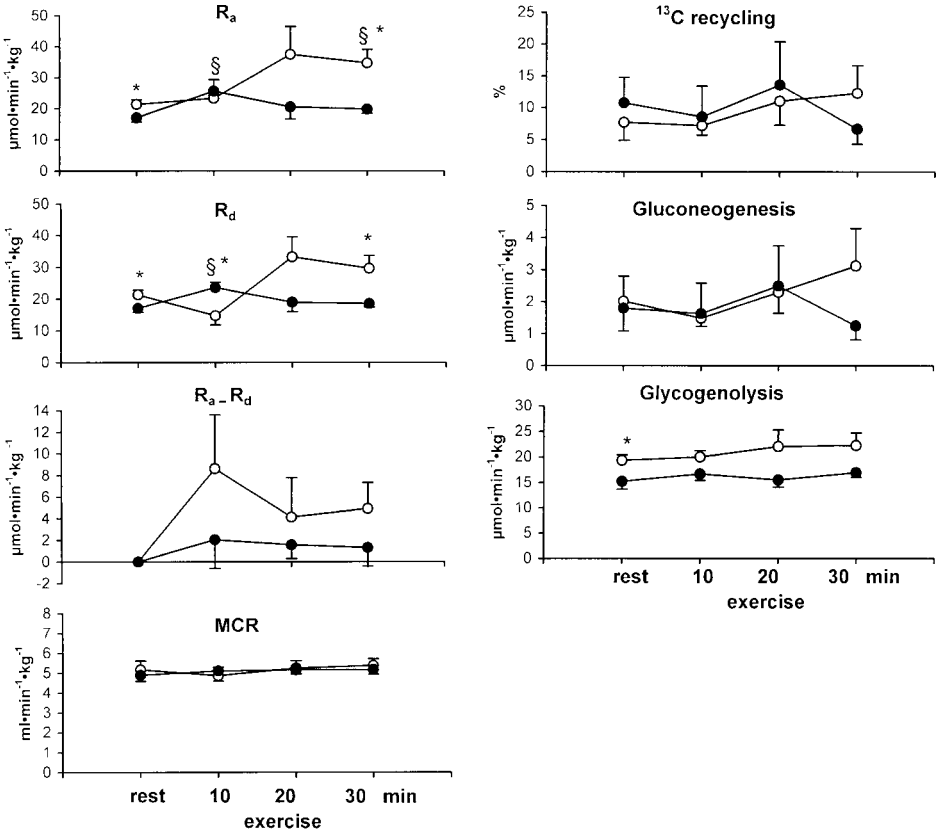

Fig 3. R_a, R_d, $R_a - R_d$, MCR, glucose recycling, gluconeogenesis, and glycogenolysis of control (○; n = 7) and shunt (●; n = 5) lambs at rest and during moderate exercise (50% of $\dot{V}O_2$ peak). Data are means ± SEM. *$P < .05$: control *v* shunt. §$P < .05$: rest *v* exercise.

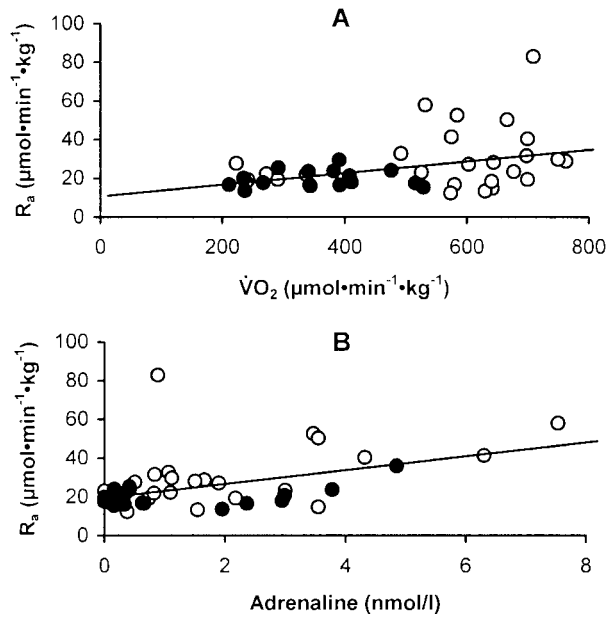

Fig 4. (A) Correlation between $\dot{V}O_2$ and R_a in control (C, ○) and shunt (SH, ●) lambs; $r = .40$; $P < .01$; $y = 10.70 + 0.03 \cdot x$. (B) Correlation between adrenaline concentration and R_a; $r = .47$; $P < .005$; $y = 20.02 + 3.61 \cdot x$.

lization during exercise. Gluconeogenesis via the Cori cycle did not increase during exercise in either group of lambs, despite an increase in the gluconeogenic precursors lactate and glycerol. The contribution of gluconeogenesis via the Cori cycle to R_a was approximately 10%. This is in agreement with the results obtained in dogs and rats during exercise.[10,29] Furthermore, it has been demonstrated that during the first hour of moderate exercise more than 90% of the increase in R_a is derived from hepatic glycogenolysis.[3]

The reason why glycogenolysis did not increase during exercise in the shunt lambs could be the low glycogen stores in the liver. That glycogen stores are important for a rise in glucose concentration during exercise, has been demonstrated.[30-32] In humans in a glycogen-depleted state, arterial glucose concentrations decreased during a bicycle test at 70% $\dot{V}O_2$ peak, whereas in a normal glycogen state the arterial glucose concentration was maintained at a similar level as at rest or showed only a slight decrease.[30] Others have shown that the increase in arterial glucose concentration and glycogenolysis was highest in those humans and rats with the highest contents of liver glycogen.[31,32] The absence in the shunt lambs of an initial decrease in R_d as occurred in the control lambs may be the result of a greater dependence of the muscles on exogenous substrates, possibly because also the muscle glycogen content is low.

Catecholamines have a stimulatory effect on hepatic glycogenolysis and gluconeogenesis or on an increase in the supply of gluconeogenic precursors.[11,12] In the control lambs, both

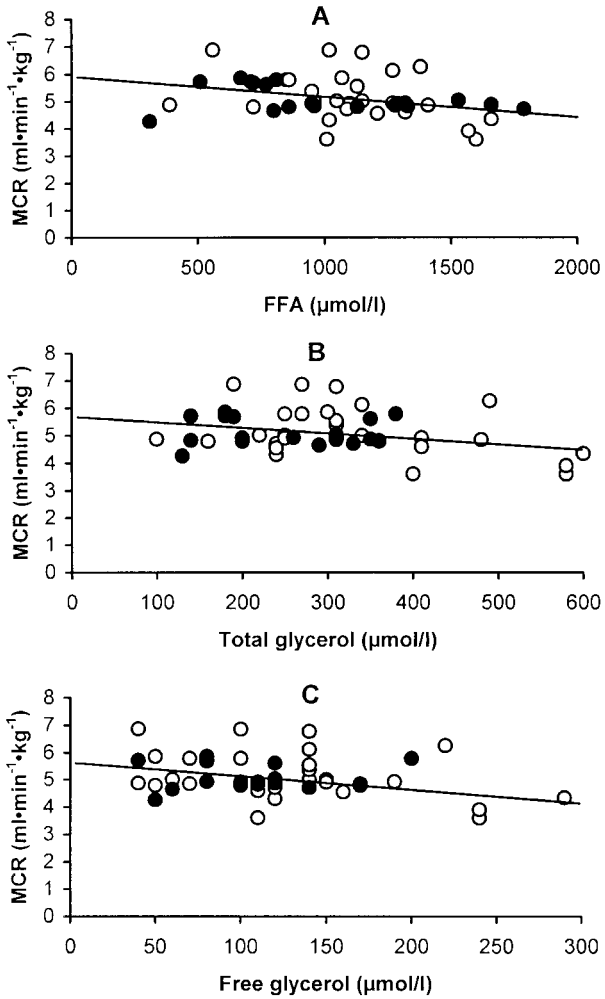

Fig 5. (A) Correlation between the MCR of glucose and the arterial concentration of FFA in control (C, ○) and shunt (SH, ●) lambs; r = -.31; $P < .05$; y = 5.88 - 0.0007 · x. (B) Correlation between MCR and arterial concentration of total glycerol; r = -.28; $P < .10$; y = 5.66 - 0.002 · x. (C) Correlation between MCR and arterial concentration of free glycerol; r = -.27; $P < .10$; y = 5.77 - 0.004 · x.

adrenaline and noradrenaline concentrations increased during exercise, whereas in the shunt lambs the adrenaline concentration showed only a slight increase and the noradrenaline concentration did not change. Furthermore, we found a correlation between R_a and the adrenaline concentration, suggesting that adrenaline plays a part in the glucoregulation. However, we did not find a statistically significant difference in adrenaline or noradrenaline concentrations between the shunt and the control lambs. Therefore, these catecholamines are not responsible for the differences found in glucose concentration, R_a of glucose, and glycogenolysis between shunt and control lambs. In a previous study[1] we have already demonstrated that neither insulin nor glucagon were responsible for these differences.

An increase in pyruvate, lactate, FFA, and free and total glycerol was found during exercise in both groups of lambs. This is in agreement with studies in humans and animals during moderate exercise.[9,11,33-35] Lipolysis seem to occur in both groups of lambs because FFA and glycerol concentrations did increase during exercise. The shunt lambs, therefore, have enough triglyceride stores to mobilize FFA and glycerol, although the percentage increase in total glycerol was lower in shunt than in control lambs. That we did not find a higher increase in FFA and glycerol concentrations due to exercise in the shunt than in the control lambs may be due to lower energy turnover reflected by the lower oxygen consumption of the shunt lambs.

The R_a of glucose was higher when the whole body oxygen consumption was higher, which means that more glucose was produced when the metabolic rate was higher. The correlation found between R_a and the arterial concentrations of glucose, lactate, FFA, and total and free glycerol is also in agreement with a higher metabolic rate. We found an inverse relationship between the arterial FFA concentration and the metabolic clearance rate of glucose, as was described in dogs by Bjorkman et al.[12] The MCR of glucose, which is a measure of glucose utilization independent of the arterial glucose concentration, is lower when the arterial concentration of FFA is higher. This is in agreement with the view that glucose and FFA are alternative fuels for the working muscles as may be expected.

A limitation of our study is that we cannot rule out the possibility that the lower glucose turnover found in the shunt lambs was caused by a lower exercise level in the shunt than in the control lambs during the moderate exercise tests. Although we had chosen a theoretically similar work load of 50% of $\dot{V}O_2$ peak, which was calculated from the values measured during a $\dot{V}O_2$ peak experiment during a fed state, a difference in work load between shunt and control lambs in the fasted state might have occurred to the extent that the shunt lambs were exercising at somewhat less than 50% of $\dot{V}O_2$ peak and the control lambs at somewhat greater than 50% of $\dot{V}O_2$ peak.

We conclude that shunt lambs have lower arterial glucose concentrations than control lambs, both at rest and during moderate exercise. We indeed found that the increase in glucose production rate in the shunt lambs was limited, which is probably due to glycogen stores. In addition, the reduced glycogenolysis rate was not offset by an increase in gluconeogenesis nor by an increase in other substrates that can be utilized by working muscles.

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