Glucose effects on lung surfactant kinetics in conscious pigs

WENJUN Z. MARTINI, OIVIND IRTUN, DAVID L. CHINKES, ROBERT E. BARROW, AND ROBERT R. WOLFE

Shriners Burns Hospital and the Departments of Surgery and Anesthesiology, The University of Texas Medical Branch, Galveston, Texas 77555

Received 24 January 2000; accepted in final form 16 May 2000

Glucose effects on lung surfactant kinetics in conscious pigs. Am J Physiol Endocrinol Metab 279: E920–E926, 2000.—The primary goal of this study was to investigate the effects of glucose infusion on surfactant phosphatidylcholine (PC) metabolic kinetics in the lungs. A new stable isotope tracer model was used in which [1,2-13C2]acetate and uniformly labeled [U-13C16]palmitate were infused in 12 normal overnight-fasted pigs to quantify lung surfactant kinetics with or without glucose infusion (24 mg·kg⁻¹·min⁻¹). With glucose infusion, the rate of surfactant PC incorporation from de novo synthesized palmitate increased from the control value of 2.1 ± 0.2 to 15.5 ± 1.9 nmol PC-bound palmitate·h⁻¹·g wet lung⁻¹ (P < 0.05), whereas the incorporation rate from plasma preformed palmitate decreased from the control value of 20.9 ± 1.9 to 11.6 ± 1.1 nmol palmitate·h⁻¹·g wet lung⁻¹ (P < 0.05). The palmitate composition in lamellar body surfactant PC increased from the control value of 61.7 ± 2.1% to 75.9 ± 0.6% (P < 0.05). The surfactant PC secretion rate decreased from the control value of 239.0 ± 66.1 to 81.9 ± 5.3 nmol PC-bound palmitate·h⁻¹·g wet lung⁻¹ (P < 0.05). We conclude that, whereas surfactant secretion was inhibited by glucose infusion, neither total surfactant PC synthesis nor the surfactant PC pool size was significantly affected due to an increased reliance on de novo synthesized fatty acids.

phosphatidylcholine; isotope; synthesis; secretion

Although there have been many in vitro investigations of the effects of glucose or insulin on surfactant production, the role of glucose and/or insulin on surfactant kinetics in vivo is still not clear. For example, Neufeld et al. (15) reported insulin inhibition of surfactant synthesis in fetal rabbit lung slices. Gross et al. (9) demonstrated a delay in the morphological maturation of the fetal rat lung in organ culture when insulin was present. On the other hand, Epstein et al. (7) found an increase in choline incorporation into phosphatidylcholine (PC) in lung slices from premature fetuses of glucose-intolerant monkeys. Wolfe et al. (20) found that insulin had a modest stimulatory effect on surfactant synthesis in the isolated perfused rat lung when palmitate concentrations were maintained constant. A direct in vivo assessment is needed to clarify the role of hyperglycemia/hyperinsulinemia on lung surfactant metabolism. This assessment is clinically important, because large amounts of glucose are commonly given as nutritional support to critically ill patients. Many of these patients have pulmonary complications, and any detrimental effect of glucose infusion on pulmonary surfactant would be reason to reevaluate the use of glucose as a primary caloric source.

The primary goal of this study was, therefore, to investigate the in vivo effects of glucose infusion on surfactant kinetics. We have recently developed a new isotope tracer methodology to quantify surfactant kinetics in conscious pigs. This model enables, for the first time, the quantification of surfactant PC synthesis from various sources of fatty acids (FA), as well as the rates of surfactant secretion, recycling, and irreversible loss. We have previously applied this methodology to quantify surfactant PC metabolic kinetics in conscious pigs in the normal postabsorptive state (14). The same isotope tracer methodology, which involves 8 h of constant infusions of [1,2-13C2]acetate and [U-13C16]palmitate, was applied in this study as in the previous study (14). Physiological hyperglycemia, with attendant hyperinsulinemia, was induced by the infusion of glucose at the rate of 24 mg·kg⁻¹·min⁻¹.

METHODS

Tracer Infusion

Twelve Yorkshire swine (K-bar live stock, Sabinal, TX), were randomly divided into the control group (n = 6, weighing 16.4 ± 2.7 kg) and the glucose group (n = 6, weighing 15.3 ± 0.5 kg) in this study. All of the animals were given ketamine (20 mg/kg im; Ketaset, Fort Dodge Laboratories, Fort Dodge, IA) for surgical catheter placement. A venous catheter was inserted into the jugular vein for isotope tracer infusion, and a second catheter was inserted via the right common carotid artery into the abdominal aorta for blood sampling. After the surgical procedure, the animals were put into slings for later isotope infusion studies. When animals awakened from the surgery anesthesia (~1–1.5 h after completion of the surgical procedure), glucose infusion was

Received 24 January 2000; accepted in final form 16 May 2000

Address for reprint requests and other correspondence: R. R. Wolfe, Shriners Burns Hospital, 815 Market St., Galveston, TX 77550 (E-mail: rwolfe@sbi.utmb.edu).

E920 0193-1849/00 $5.00 Copyright © 2000 the American Physiological Society http://www.ajpendo.org
started in the animals in the glucose group at the rate of 24 mg·kg⁻¹·min⁻¹ until the end of the study. Saline (0.9% N) was infused at the same volume rate in the animals in the control group. Blood glucose concentrations were measured every hour in both groups throughout the entire study. Two hours after the start of glucose (or saline) infusion, a background blood sample was taken, and a constant infusion of [1,2-¹³C₂]acetate (99% enriched, 95.5 molar percent excess (MPE) [1,2-¹³C₂]acetate; Isotec, Miamisburg, OH; 24 μmol·kg⁻¹·min⁻¹) and uniformly labeled [U-¹³C₁₆]palmitate (99% enriched, 76.6 MPE [¹³C₁₆]palmitate; Isotec; 0.16 μmol·kg⁻¹·min⁻¹) was given for 8 h via calibrated syringe pumps (Harvard Apparatus, Natick, MA). The start of the isotope infusion was considered time 0 in Fig. 1, A and B. Blood samples (10 ml) were drawn each hour throughout the 8-h isotope infusion. After collection of the last hourly infusion sample, the animals were killed with ketamine (10 mg/kg iv) followed by a solution of saturated potassium chloride (2 mg/kg iv). The lungs were quickly removed, weighed (191.9 ± 32.9 g in control group and 176.8 ± 5.5 g in glucose group), and cooled for bronchoalveolar lavage (BAL) and tissue sampling. This experimental protocol was approved by the Animal Care and Use Committee of the University of Texas Medical Branch (90–09–103–1).

BAL

The right lung was isolated, weighed, vacuum degassed, and suspended in a Plexiglas vacuum chamber via the trachea cannula, which passed through the top of the chamber to two 150-ml syringes connected by a three-way valve. A subatmospheric chamber pressure was externally applied to the lung, and the lung was filled with fluid consisting of normal saline plus 0.01 M Tris buffer through the syringes. This fluid was then withdrawn from the lung into the syringes by application of a positive pressure to the lung along with gentle airway suction. To ensure good mixing of BAL, the fluid collected in the syringes was pushed into the lung again and then withdrawn back into the syringes. The BAL was then transferred into a collecting flask. The entire procedure was repeated six times, so that six BAL washes were collected from each animal. The volume of each BAL wash was measured for the calculation of total PC content in BAL. Any fluid leaking from the lungs was collected and added to the appropriate BAL wash. The total alveolar PC pool size was calculated as the sum of the PC content from these six BAL washes.

Sample Analysis

Alveolar surfactant isolation. Lung surfactant from BAL was isolated according to a previously described technique (4). The BAL was filtered through gauze, and the volume was measured. An aliquot of BAL was centrifuged at 160 g for 20 min to remove intact cells and cellular debris. The supernatant was collected and centrifuged in an ultracentrifuge at 100,000 g for 1 h at 4°C (Model XL-80 Beckman Ultracentrifuge, SW 40 Ti rotor, Beckman Instruments, Palo Alto, CA). The precipitate pellet was stored at −70°C for lipid extraction.

Lamellar body isolation. After BAL collection, 0.5 g of lung tissue was minced and homogenized in 1 M sucrose for 3 min with a Brinkman Homogenizer, Brinkman Instrument, Westbury, NY. The homogenate was filtered, and the volume was adjusted to 5 ml with 1 M sucrose. A discontinuous sucrose density gradient from 0.9 to 0.2 M was loaded on top of the homogenates, as described by Duck-Chong (6). The gradients were centrifuged at 200 g for 15 min and then at 116,000 g for 3 h at 4°C. The lamellar bodies sedimented in the 0.45 M sucrose layer were identified as a white opaque band similar to that described by Duck-Chong and by Young et al. (23). This band was aspirated, quantified by volume, and stored at −70°C for lipid extraction.

PC isolation from surfactant. The lipids from the lamellar bodies and from BAL were extracted with a mixture of hydrochloric acid, heptane, and 2-propanol (1:10:40 by vol). The upper layer was aspirated and dried under nitrogen. The lipid extract was separated by thin-layer chromatography on a silica gel plate with a two-solvent system of 1) chloroform-methanol-water (65:30:5 by volume), and 2) heptane-ethyl ether-acetic glacial acid (80:20:2 by vol). The PC location was identified by iodine staining, scraped from the gel plate, and dissolved in 1 ml chloroform. A 250-μl aliquot was used for PC-bound palmitate enrichment measurements, a 250-μl aliquot was used for PC-bound FA composition determinations, and a 500-μl aliquot was used for phosphorus analysis according to the method described by Bartlett (5). [¹⁴C]dipalmitoyl PC was used to monitor the recovery (54 ± 2%) of this isolation procedure. The total PC contents in the lamellar bodies and BAL were used for calculation of surfactant PC synthetic rates and secretion rates, respectively.

FA isolation from plasma and PC from BAL and lamellar bodies. Plasma (500 μl each) samples were extracted by use of a mixture of hydrochloric acid, heptane, and 2-propanol (1:10:40 by vol). The upper layer was aspirated and dried under nitrogen. The free fatty acid (FFA) extract was isolated by thin-layer chromatography using a mixture of heptane, glacial acetic acid, and ethyl ether (80:20:20 by vol). The FA location was identified by iodine staining, scraped, and stored at −70°C for the determination of isotope enrichment and composition.

Samples of PC isolated from the lamellar bodies and BAL were hydrolyzed for 30 min with a mixture of 0.6 ml isopropanol and 0.3 ml potassium hydroxide (0.1 N) kept at 110°C. The FA from the hydrolyzed tissue were isolated by extraction with 0.3 ml HCl (0.1 N), 0.2 ml H₂O, 1.4 ml methanol, and 3 ml chloroform.

FA enrichment measurement. The FA isolated from PC in BAL, lamellar bodies, and plasma were methylated with boron trifluoride in methanol, and the isotopic enrichment of

\[ \text{E921GLUCOSE AND SURFACTANT} \]
palmitate methyl ester was determined by gas chromatography-mass spectrometry (GC-MS, model 5972, Hewlett-Packard) in the electron impact ionization mode: ions of mass-to-charge ratio (m/z) 270(m + 0), 271(m + 1), 272(m + 2), 273(m + 3), 274(m + 4), and 286(m + 16) were selectively measured.

Concentration and composition. Plasma FFA composition and concentration from each hourly sampling were determined by HPLC (Waters 2690, Milford, MA), following the procedure described by Uji et al. (22). Heptadecanoic acid was used as an internal standard. FA compositions in lamellar body PC and alveolar surface PC from each animal were also measured by HPLC.

Plasma very low density lipoprotein-triglyceride isolation. Plasma very low density lipoprotein (VLDL) was isolated from 3 ml of plasma by overlaying the plasma with 0.9% normal saline with a density of 1.006 and subsequent ultracentrifugation at 50,000 rpm (171,500 g) for 20 h at 4°C. After ultracentrifugation, the VLDL was carefully removed along with the density solution by slicing the tube into sections (10). VLDL-triglyceride (VLDL-TG)-bound FA were isolated and measured following the same procedures as in PC-bound FA.

Blood glucose and plasma insulin concentrations were measured on hourly samples. Blood glucose was measured with a 2300 STAT analyzer (Yellow Spring Instruments, Yellow Springs, OH), and plasma insulin concentration was determined by a radioimmunoassay method (INCSTAR, Stillwater, MN).

Calculation
The calculations of surfactant kinetics have been described in detail previously (14). Briefly, surfactant fractional synthetic rate was calculated as

\[
FSR_{syn} = \frac{E_{EA}(t)}{F_{EL}(t)} - \frac{E_{EA}(t) - t}{2}
\]

(1)

where \( t \) is the duration of the isotope infusion, \( E_{EA}(t) \) is surfactant PC-bound palmitate enrichment in lamellar bodies at the end of the infusion, and \( E_{EL}(t) \) is precursor enrichment in the steady state during infusion. In the calculation of surfactant PC incorporation from plasma palmitate, \( E_{EL}(t) \) is the plasma palmitate enrichment. In the calculation of the incorporation from de novo synthesized palmitate, \( E_{EA}(t) \) is calculated from isotopomer distribution analysis (11), where

\[
E_{EA}(t) = 8p(1 - p)^t
\]

(2)

\[
p = \frac{2 TTR(M + 4) / TTR(M + 2)}{7 + 2 TTR(M + 4) / TTR(M + 2)}
\]

(3)

TTR(\(M + 2\)) [tracer-to-tracee ratio (TTR) of the ion \( m + 2\)] and TTR(\(M + 4\)) (TTR of the ion \( m + 4\)) are the enrichments of doubly labeled palmitate and quadruply labeled palmitate, respectively, bound to lamellar body PC. We found that plasma palmitate is a major contributor of lamellar body PC (14). For this reason, we would expect that palmitate synthesized in tissues other than the lung would be incorporated into lamellar body PC via plasma palmitate, especially after glucose infusion. To account for this, the enrichment of \((m + 2)\)-labeled palmitate in lamellar body PC that came from plasma palmitate was deduced by multiplying the enrichment of TTR(\(m + 16\)) in lamellar body PC (which only comes from plasma palmitate) by the ratio of the TTR(\(m + 2\)) and TTR(\(m + 16\)) enrichments in plasma palmitate. In a similar manner, the enrichment of TTR(\(m + 4\))-labeled palmitate in lamellar body PC that came from plasma palmitate was deduced by multiplying the enrichment of TTR(\(m + 16\)) in lamellar body PC (which only comes from plasma palmitate) by the ratio of the TTR(\(m + 4\)) and TTR(\(m + 16\)) enrichments, respectively, to give the enrichment of lamellar body PC that came from de novo synthesis in the lung tissue and VLDL-TG. From a rough estimate of the contribution of labeled VLDL-TG (see DISCUSSION), it was concluded that very little labeled lamellar body PC came from labeled VLDL-TG. We then calculated the incorporation of de novo synthesized FA from the lung tissue into lamellar body PC by use of the calculated values for TTR(\(m + 2\)) and TTR(\(m + 4\)) in Eq. 3.

The fractional synthetic rate (FSR) from de novo synthesized FA synthesized from places other than lung tissue was calculated as above, except for use of the TTR(\(m + 2\)) and TTR(\(m + 4\)) labels that came from plasma palmitate rather than from the lung tissue.

The absolute synthesis rate is calculated by multiplying the FSR(\(t\)) with the pool size of lamellar body PC-bound palmitate.

The fractional secretion rate is calculated as

\[
FSR_{sec} = \frac{E_{EL}(t)}{E_{EL}(t) - E_{NO}(t)/2}
\]

(4)

where \( E_{EL}(t) \) and \( E_{NO}(t) \) are PC-bound palmitate enrichments at the end of the infusion in the alveolar surface pool and lamellar body pool, respectively. As before, the absolute rate of secretion is calculated by multiplying FSR(\(sec\)) with the pool size of alveolar surface PC-bound palmitate.

We assume that a physiological steady state exists during the time of measurements. In this case, the rate of irreversible loss of alveolar surface PC must be equal to the sum of the synthetic rates from plasma preformed palmitate and de novo synthesized palmitate. The recycling rate is calculated by subtracting the secretion rate from the rate of irreversible loss. Thus all the parameters represented in Fig. 2 can be quantified.

Statistical Analysis
All results are expressed as means ± SE. Data from the control group have been published previously (14). To evaluate the effects of treatment on glucose and insulin concentrations, a two-way repeated-measures ANOVA was performed with the factors time and treatment. Post hoc comparisons at each time point were accomplished by use of Tukey’s test. Other comparisons between the control and glucose groups were made with Student’s t-test. Statistical significance is set at the 0.05 level.

RESULTS
Blood Measurements
A physiological steady state was achieved in each pig during the 8-h isotope infusion, as indicated by the constant concentrations of plasma FFA, VLDL-TG, glucose, and insulin. The concentrations of blood glucose and plasma insulin in the control and glucose groups during the eight hourly isotope infusion are shown in Fig. 1, A and B, respectively. The average blood glucose level from the eight hourly samples was increased from the control value of 120 ± 9 to 148 ± 14 mg/dl (\( P < 0.05 \)) during the glucose infusion. The average plasma insulin level from the eight hourly
samples was also increased during the glucose infusion from the control value of 7.0 ± 0.6 to 17.9 ± 1.5 µIU/ml (P < 0.05).

During the 8-h isotope infusion, arterial blood gas measurements in the glucose group were: PaO2, 94 ± 4 Torr; PaCO2, 41.3 ± 2.6 Torr; pH, 7.499 ± 0.030; and O2 content, 10.0 ± 0.4 vol%. There were no significant differences between the control and the glucose groups. With glucose infusion, the average of plasma total fatty acid concentration from eight hourly samples taken during the 8-h isotope infusion was decreased from the control value of 615 ± 73 to 104 ± 2 nmol/ml (P < 0.05). The average plasma palmitate concentration was also decreased from the control value of 183 ± 6 to 21 ± 35 nmol/ml (Table 1). However, the average proportionate contribution of plasma palmitate to the total plasma FFA increased from 29.7 ± 0.4% (control value) to 33.5 ± 0.3% (P < 0.05), whereas the contributions of plasma oleate (18:1) were significantly decreased (Table 1).

**Table 1. Plasma free fatty acid composition and concentration**

<table>
<thead>
<tr>
<th>Composition, %</th>
<th>Palmitate</th>
<th>Stearate</th>
<th>Oleate</th>
<th>Linoleate</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control group</td>
<td>29.7 ± 0.4</td>
<td>11.3 ± 0.3</td>
<td>46.5 ± 0.8</td>
<td>11.8 ± 0.6</td>
<td>99.3 ± 0.9</td>
</tr>
<tr>
<td>Glucose group</td>
<td>33.5 ± 0.3*</td>
<td>26.8 ± 0.5*</td>
<td>15.4 ± 0.3*</td>
<td>24.2 ± 0.5*</td>
<td>99.9 ± 0.6</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Concentration, nmol/ml</th>
<th>Palmitate</th>
<th>Stearate</th>
<th>Oleate</th>
<th>Linoleate</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control group</td>
<td>183 ± 21</td>
<td>69 ± 10</td>
<td>286 ± 37</td>
<td>73 ± 14</td>
<td>615 ± 73</td>
</tr>
<tr>
<td>Glucose group</td>
<td>35 ± 1*</td>
<td>28 ± 1*</td>
<td>17 ± 1*</td>
<td>16 ± 0.1*</td>
<td>104 ± 2*</td>
</tr>
</tbody>
</table>

Values are means ± SE. Data are the averages of measurements from hourly samples taken during the 8-h isotope infusion. *P < 0.05 between the control and glucose groups.

**Table 2. Fatty acid composition in surfactant PC**

<table>
<thead>
<tr>
<th>Composition</th>
<th>Palmitate</th>
<th>Stearate</th>
<th>Oleate</th>
<th>Linoleate</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lamellar body</td>
<td>61.7 ± 2.1</td>
<td>14.1 ± 1.7</td>
<td>19.1 ± 2.9</td>
<td>5.1 ± 0.9</td>
</tr>
<tr>
<td>Control group</td>
<td>75.9 ± 0.8*</td>
<td>13.8 ± 1.1*</td>
<td>5.6 ± 0.7*</td>
<td>3.4 ± 0.3*</td>
</tr>
<tr>
<td>Glucose group</td>
<td>83.4 ± 0.8*</td>
<td>9.6 ± 0.6*</td>
<td>4.0 ± 0.7*</td>
<td>2.6 ± 0.2*</td>
</tr>
</tbody>
</table>

Values are means ± SE of 6 animals in each group. PC, phosphatidylcholine. *P < 0.05 between the control and glucose groups.

**Pool Sizes and FA Composition of Surfactant**

With glucose infusion, the surfactant PC pool size in the lamellar bodies was increased from the control value of 1.14 ± 0.02 to 1.41 ± 0.04 µmol/g wet lung (P < 0.05), and the PC pool size in the alveolar surface was decreased from the control value of 1.46 ± 0.12 to 1.09 ± 0.07 µmol/g wet lung (P < 0.05). The total surfactant PC pool sizes in lamellar bodies and alveolar surface remained the same. The proportionate contributions of palmitate to the lamellar body PC (75.9 ± 0.6% of FA) and in the alveolar surface PC (83.4 ± 0.8% of FA) were significantly increased compared with the control values of 61.7 ± 2.1 and 71.9 ± 1.4%, respectively (Table 2).

**Rates of Synthesis and Loss**

Plasma palmitate enrichment [TTR(m + 16), Ep] reached an isotopic steady state (0.131 ± 0.011) within 2 h of [U,13C]palmitate infusion. At the end of the 8-h isotope infusion, the enrichments of TTR(m + 16) from lamellar PC-bound palmitate and alveolar surface PC-bound palmitate were 0.006 ± 0.000 and 0.002 ± 0.000, respectively. The enrichments of TTR(m + 2) and TTR(m + 4) from lamellar body PC-bound palmitate at the end of the infusion were 0.010 ± 0.001 and 0.013 ± 0.002, respectively. The calculated precursor enrichment (p) for palmitate that was de novo synthesized in lung tissue was 0.386 ± 0.036.

With glucose infusion, the FSR of PC incorporation from de novo synthesized palmitate in lung tissue was increased from the control value of 0.14 ± 0.02 to 0.55 ± 0.07%/h (P < 0.05). At the same time, the FSR from plasma preformed palmitate was decreased from the control value of 1.44 ± 0.13 to 0.54 ± 0.05%/h (P < 0.05) by 10.2 ± 0.33 on October 29, 2017 http://ajpendo.physiology.org/ Downloaded from
The synthetic rate from palmitate that was de novo synthesized in lung tissue was increased from the control value of 1.8 ± 0.2 to 12.0 ± 1.9 nmol PC-bound palmitate·h⁻¹·g wet lung⁻¹ (P < 0.05), and the synthetic rate from palmitate that was de novo synthesized in other tissues was increased from the control value of 0.3 ± 0.0 to 3.5 ± 0.3 nmol PC-bound palmitate·h⁻¹·g wet lung⁻¹ (P < 0.05). The synthetic rate from plasma preformed palmitate was decreased from the control value of 20.9 ± 1.9 to 11.6 ± 1.1 nmol PC-bound palmitate·h⁻¹·g wet lung⁻¹ (P < 0.05). However, the relative synthetic rate from plasma preformed palmitate, which was calculated by dividing the synthetic rate by plasma palmitate concentration, was significantly increased from the control value of 0.111 ± 0.001 to 0.354 ± 0.021 ml·h⁻¹·g wet lung⁻¹ (P < 0.05).

Despite the significant changes in synthetic rates from de novo synthesized palmitate and from plasma palmitate, the total synthetic rates, which were equal to irreversible loss rates, were similar between the control value (22.9 ± 2.1) and that with glucose infusion (27.1 ± 2.4 nmol PC-bound palmitate·h⁻¹·g wet lung⁻¹).

**Contribution of De Novo Synthesized FA of Different Origins to PC Synthesis**

The FSR from de novo synthesized FA synthesized in lung tissue with glucose infusion was 0.55 ± 0.07%/h, whereas the FSR from FA synthesized elsewhere (other than in lung and liver) was 0.16 ± 0.02%/h. Therefore, lung tissue was the predominant site of de novo synthesized FA incorporated into surfactant PC.

**Rates of Secretion and Recycling**

With glucose infusion, the calculated FSR (FSRsec) was decreased from the control value of 12.3 ± 2.0 to 4.5 ± 0.5%/h (P < 0.05). The secretion rate was decreased from the control value of 239.0 ± 26.1 to 81.9 ± 5.3 nmol PC-bound palmitate·h⁻¹·g wet lung⁻¹ (P < 0.05). The recycling rate was also decreased from the control value of 215.9 ± 27.3 to 54.7 ± 4.6 nmol PC-bound palmitate·h⁻¹·g wet lung⁻¹ (P < 0.05). About 67% of secreted surfactant PC was recycled for reutilization, compared with 90% in the control value. All of the calculated kinetics are summarized in Fig. 2.

**DISCUSSION**

We recently developed a new tracer model to quantify surfactant PC kinetics in conscious animals (14). We found that, in the postabsorptive state, plasma was the primary source of FA used for surfactant PC synthesis (14). In the current study we found that 8 h of high-dose glucose infusion changed the proportional contribution of FA for surfactant PC synthesis, with de novo synthesized fatty acids playing a more predominant role. Because palmitate is the principal product of de novo synthesis, the percentage of palmitate in surfactant PC increased significantly during glucose infusion. A higher percentage of palmitate in surfactant PC should improve its surfactant function (21). Furthermore, although surfactant secretion and recycling were markedly decreased by glucose infusion, the rate of total surfactant synthesis was not affected. Consequently, despite significant changes in several aspects of surfactant kinetics, glucose did not affect the sum total of surfactant pools in the lamellar bodies and alveolar surface. Because the total surfactant pool size did not change and the change in the PC FA composition would likely be favorable with regard to function, we are unable to identify a role of hyperglycemia/hyperinsulinemia in causing pulmonary insufficiency via an effect on lung surfactant. To the contrary, our results indicate that, in clinical situations in which pulmonary function may be compromised, high-dose glucose ingestion may have a beneficial effect on pulmonary surfactant.

The decreased importance of plasma FA (specifically palmitate) as precursor for surfactant PC synthesis during glucose infusion can be attributed to the antilipolytic effect of insulin (12). Inhibition of lipolysis caused plasma FA to decrease to <20% of the control value. Nonetheless, plasma FFA remained the predominant source of FA for lung PC synthesis. This reflected a significant stimulation of the efficiency of synthesis of lung PC from plasma FFA as reflected by the significant increase in the rate of incorporation of plasma FFA when normalized for the prevailing plasma concentration. This finding is consistent with our previous study in the perfused rat lung (20), in which we found that the rate of incorporation of plasma palmitate into surfactant was stimulated by insulin when the palmitate concentration in the perfusate was maintained constant. Thus it appears that insulin does not exert a direct inhibitory effect on surfactant synthesis.

At the same time that plasma FA availability decreased, the incorporation of de novo fatty acids into pulmonary PC increased. Because palmitate is the product of de novo synthesis (8), the percentage of the contribution of palmitate to the newly synthesized surfactant PC also increased. The increased importance of de novo synthesized FA as precursors for PC synthesis likely reflects a stimulation of de novo synthesis by glucose. We previously found in normal human volunteers that the administration of a comparable amount of glucose for 24 h caused a significant increase in the synthesis of FA in the liver as well as total fat synthesis (1). Furthermore, in that study, we observed that the rates of fat synthesis increased severalfold more after 4 days of continuous high-dose glucose (1). It is thus likely that the increased incorporation of de novo synthesized FA into pulmonary surfactant PC observed in this study would have increased even further with more prolonged administration of glucose in the content of enteral or parenteral nutrition.

Our methodology enables the distinction of PC synthesis from plasma FFA that was preformed, as opposed to newly synthesized FA. Approximately 35% of the plasma FFA arose from de novo synthesis, a figure that may seem surprisingly high, because in human subjects, FA synthesis has been thought to occur...
mainly in the liver (3). However, in a recent study, we showed that, during high-dose glucose infusion in normal human volunteers, most de novo FA synthesis could not be explained by fatty acids synthesized in the liver and secreted as VLDL-TG (2). The accuracy of our estimation showing that a significant portion of the plasma FFA arose from de novo synthesis is supported by the significant increase in the proportionate contribution of palmitate to the total FFA (Table 2). Of course, the proportionate contribution of de novo synthesized FA to the plasma FFA was increased not only by the increased synthesis of palmitate but also by the suppression of lipolysis and subsequent release of preformed FFA into the blood from adipose tissue.

Whereas our methodology enables distinction between the incorporation of plasma preformed FA and de novo synthesized FA into lung PC, the methodology does not definitively identify all the sites of de novo synthesized FA used for PC synthesis. However, we can distinguish between de novo synthesis in the lung and in other tissues; we found that the lung was the predominant site. The newly synthesized FA that were produced elsewhere would have to be transported to the lungs via the blood before incorporation into surfactant. We have discussed above the quantitative contribution of de novo synthesized plasma FA. The only other potentially significant source of circulating FA would be in VLDL-TG; however, this source is not likely a major contribution to lung PC FA. By measuring the secretion rates of VLDL-TG from de novo synthesized FA in the current study as described previously (1), we found that glucose increased the rate from 0.28 ± 0.06 to 7.34 ± 2.55 μmol VLDL-TG·kg⁻¹·day⁻¹. This magnitude of increased VLDL-TG secretion of newly synthesized FA is consistent with our previous results in humans (1) and indicates that hepatic FA synthesis can be markedly activated by glucose infusion. The FA in circulating VLDL-TG are theoretically available to lung tissue. However, the rate of secretion of VLDL-TG is not high in relation to lung PC synthesis. Thus the total delivery of de novo synthesized palmitate to the lungs in the form of VLDL-TG was 4.32 μmol/h during glucose infusion. At the same time, de novo synthesized palmitate was incorporated into pulmonary surfactant PC at the rate of 3.0 μmol/h. Even if as much as 10% of VLDL-TG were cleared by the lungs (which is a considerable overestimate of the likely value) and all of the resulting palmitate were incorporated into surfactant PC (again an unlikely exaggeration), the contribution of this source of palmitate would still be only 14% of the total de novo synthesized palmitate. Considering the liberal overestimates of both VLDL-TG extraction and efficiency of incorporation into lung surfactant PC used in this example, it is safe to conclude that circulating VLDL-TG are not an important source of de novo synthesized FA for lung surfactant PC. It is therefore reasonable to conclude that, during glucose infusion, the lung is the major site of synthesis of de novo synthesized palmitate that is subsequently incorporated into surfactant PC.

We assume that surfactant PC enrichment increases linearly and starts at the beginning of the 8-h isotope infusion (time 0). Our previous studies with different infusion time periods have shown a linear increase in surfactant PC enrichment (14). Although a delay in appearance of isotope label in surfactant PC might be expected, our previous data (14) have shown that this delay was insignificant compared with the 8-h infusion interval we used. Therefore, it is reasonable to assume that surfactant PC enrichment starts at time 0 and increases linearly.

The formula we used to calculate the fractional synthesis of lamellar body PC assumes that there is no recycling of label from the alveolar surface PC pool to the lamellar body PC pool. Our results show that recycling rates of unlabeled alveolar PC to the lamellar body PC pool are 2–10 times greater than the rates of synthesis of lamellar body PC, which raises a concern for this assumption. However, because the enrichment of the precursor for synthesis is much greater than the enrichment of the alveolar surface PC over the 8-h infusion, the amount of label in the lamellar body PC pool comes predominantly from synthesis. Therefore, the effect of tracer recycling on PC synthesis is not significant. We can express this effect of recycling on FSRsyn in quantitative terms. In our previous study (14), we demonstrated that, if the recycling does occur, the formula to calculate the FSR is

\[
\text{FSR}_{\text{syn}} = \frac{E_L(t_2) - E_L(t_1) - \frac{F_{\text{LA}}}{Q_L} \int_{t_1}^{t_2} (E_A - E_L) \, dt}{\int_{t_1}^{t_2} (E_P - E_L) \, dt}
\]

where \(E_L\) is the lamellar body PC enrichment, \(E_P\) is the plasma palmitate enrichment, \(E_A\) is the alveolar PC enrichment, \(F_{\text{LA}}\) is the rate of recycling, and \(Q_L\) is the lamellar body pool size. The above formula is the same as the formula we used except for the additional term that accounts for recycling. The rate of recycling is the only variable in the above equation that we have not directly measured. However, we can use the rate of PC secretion from the lamellar body pool to the alveolar pool as an upper bound of the rate of recycling. (The recycling issue does not affect the calculation of the FSR of PC, because the only precursor for alveolar PC is lamellar body PC.) In the control group, the term \(E_L(t_2) - E_L(t_1)\) is 0.003, and the term \(\frac{F_{\text{LA}}}{Q_L} \int_{t_1}^{t_2} (E_L - E_A) \, dt < 239/1,140 \times (0.003 - 0.002)/2 = 0.0001\), so the maximum error in assuming no recycling is 0.0001/0.003 = 3.3%. In the glucose group, the maximum error is 54.7/1,410 = 0.006/0.002/2/0.006 = 1.3%. Therefore, our assumption of no tracer recycling during the 8-h infusion is reasonably accurate.

Earlier papers (13, 16, and 18) have also concluded that palmitate in lung surfactant PC is derived from de novo synthesis within the lung. However, the important observation in the current study is that, when the relative pathways are quantified, it is only in the ex-
extremely lipogenic state of chronic high-dose glucose infusion that lung de novo synthesis plays an important role. Furthermore, even in this extreme circumstance of hyperglycemia/hyperinsulinemia, the contribution from plasma preformed palmitate was still similar to that from de novo synthesized palmitate in the lung (Fig. 2), even though the availability of FFA was low as a consequence of suppressed lipolysis. Furthermore, the persistent utilization of plasma FFA was striking when the relatively low proportion of palmitate in plasma FFA (∼30%) is considered in light of the composition of lung surfactant PC (∼70% palmitate). This reflects a high degree of selectivity in the synthesis process. Thus, although an increase in palmitate availability via the synthetic pathway resulted in a proportionate change in the relative amounts of individual FA in PC (i.e., increased proportion of palmitate), it is evident that precursor availability is of only limited importance in determining the composition of PC. Rather, it appears likely that composition is determined largely at the site of PC synthesis, presumably through a deacylation-reacylation pathway (17).

The findings of this paper are relevant to the clinical administration of high-dose glucose in the context of nutritional support. It is common in such patients that pulmonary problems, including decreased lung compliance, impair recovery. Past publications claiming an inhibitory effect of insulin on surfactant synthesis (9, 15) combined with increased CO₂ production resulting from FA synthesis (19) have led to the general recommendation to limit glucose intake in critically ill patients. Our findings lend no support to this perspective. In fact, the increased proportion of palmitate in lung PC may even improve its surfactant function. On the other hand, we found that glucose infusion limited both secretion and recycling of surfactant PC. This occurred in the absence of any change in the total surfactant pool size, so it is unclear whether there is a physiological consequence of decreased secretion/recycling. Thus, whereas in the absence of any compelling evidence to the contrary it is reasonable to conclude that nutritional support with glucose/carbohydrate presents no detriment to surfactant function, further evidence regarding the physiological significance of altered secretion/recycling over a prolonged period of time may cause a revision of this conclusion. With regard to respiratory distress in infants of diabetic mothers, our results indicate that it is unlikely that insulin per se or hyperglycemia directly limits surfactant synthesis.

We thank Lillian Traber and colleagues for their guidance in performing this study.

This investigation was supported by National Institutes of Health Grant 2RO1 DK-34817–15 and Shriners Hospital Grants 8050 and 8490.

REFERENCES