

# Transfer of Insulin Lispro Across the Human Placenta

## In vitro perfusion studies

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**OBJECTIVE** — Insulin lispro (Humalog), a human insulin analog, has a more rapid onset, earlier peak, and shorter duration of glucose lowering activity than regular human insulin. However, it is not known whether insulin lispro crosses the human placenta and reaches the fetus. Therefore, the objective of the present study was to examine whether insulin lispro crosses the placenta using the technique of perfusing a human placental lobule in vitro.

**RESEARCH DESIGN AND METHODS** — Term human placentae from uncomplicated pregnancies were obtained immediately after delivery. Insulin lispro, at concentrations ranging from 100 to 1000  $\mu\text{U}/\text{ml}$ , was introduced into the maternal reservoir. The maternal side of the placenta was perfused with constant concentration of lispro insulin; the fetal circulation was closed. Samples were drawn from both the maternal and fetal circulations at regular intervals. The appearance of insulin lispro in the fetal circulation was analyzed by a specific radioimmunoassay.

**RESULTS** — No placental transfer of lispro could be detected during perfusion with 100 and 200  $\mu\text{U}/\text{ml}$ . In contrast, there was a small concentration-dependent transfer to the fetus at concentrations of 580  $\mu\text{U}/\text{ml}$  and higher, detectable after at least an hour of constant concentration of insulin lispro during perfusion. The rate of placental transfer was  $0.019 \mu\text{U} \cdot \text{min}^{-1} \cdot \text{g tissue}^{-1}$  at maternal levels of 580  $\mu\text{U}/\text{ml}$  and  $0.045 \mu\text{U} \cdot \text{min}^{-1} \cdot \text{g}^{-1}$  tissue at maternal levels of 1000  $\mu\text{U}/\text{ml}$ . Measuring lispro levels in 11 pregnant women revealed that a dose of 50 units may achieve serum concentrations  $>200 \mu\text{U}/\text{ml}$  with apparent linear correlation between dose and levels.

**CONCLUSIONS** — Insulin lispro is not likely to cross the placenta at a single standard dose. This study suggests that insulin lispro is unlikely to reach or harm the unborn baby.

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Insulin lispro is an insulin analog in which two amino acids, lysine and proline, on the B-chain are reversed. This results in a weaker tendency for self-association after subcutaneous injection, allowing for more rapid absorption of the insulin, faster onset, and shorter duration

of action when compared with regular human insulin (1,2). Studies of insulin lispro have confirmed that postprandial glucose levels are lower using insulin lispro compared with regular human insulin, independent of HbA<sub>1c</sub> level (2). This may be important in pregnancy where

high postprandial glucose levels can lead to fetal macrosomia (3). Adjustment of insulin therapy in gestational diabetes to normalize postprandial glucose levels leads to normalized birth weight and lower rates of cesarean sections (4). There is also evidence that the use of insulin lispro is associated with a reduction in the frequency of hypoglycemia compared with regular human insulin (5). In a meta-analysis of eight large clinical trials comparing insulin lispro to regular human insulin, the frequency of severe hypoglycemic episodes was lower in patients taking insulin lispro (6). There is an increased risk of severe hypoglycemic episodes in pregnancy (7), hence strategies to reduce this risk now include the use of insulin lispro. In a randomized double-blind trial of insulin lispro versus human regular insulin using a continuous subcutaneous insulin infusion, patients using insulin lispro had significantly lower HbA<sub>1c</sub> levels (8).

Whereas early in vitro studies suggested that insulin does not cross the human placenta (9), a subsequent placental perfusion study demonstrated that a small amount of human insulin, representing 1–5% of the insulin concentration in the maternal artery, was transferred from the maternal to the fetal circulation (10). The concentrations of insulin used in that experiment (59, 104, 448, and 1,198  $\mu\text{U}/\text{ml}$ ) would, based on an assumption of linear pharmacokinetics, correspond to peak serum insulin levels achieved after subcutaneous injection doses of ~14, 24, 104, and 278 units of insulin, respectively (1). In addition, there is some evidence that beef-pork–insulin antibody complexes can cross the placenta leading to neonatal macrosomia (11). Currently, there is limited information on the placental transfer of insulin lispro. The potential consequences of placental transfer include the risk of neonatal hyperinsulinemia and hypoglycemia. Animal studies suggest that hypoglycemia may cause teratogenesis (12). There has been a recent report of congenital anomalies in the off-

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**Abbreviations:** hCG, human chorionic gonadotrophin.

A table elsewhere in this issue shows conventional and Système International (SI) units and conversion factors for many substances.

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spring of two women taking insulin lispro throughout pregnancy, despite optimal glycemic control (13). However, no causal relation with insulin lispro was established. In studies with insulin lispro in pregnant rats and rabbits, no evidence of impaired fertility or harm to the fetuses was observed with doses as high as four times the average dose in humans (14). The objective of the present study was to examine whether insulin lispro crosses the placenta using the technique of perfusing a human placental lobule in vitro.

## RESEARCH DESIGN AND METHODS

### Study design

The eight human placentae were obtained after vaginal or cesarean section delivery from uncomplicated term pregnancies and transported to the laboratory in heparinized ice-cold PBS. Independent maternal and fetal circulations were established to a peripheral placental lobule within 30 min of the delivery of the infant, as previously described by our laboratory (15).

After residual blood was cleared out of the vessels and intervillous space, a 1-h "closed" (recirculated) circuit control period preceded the experimental period. During the control period, glucose and oxygen consumption and lactate and human chorionic gonadotrophin (hCG) production were measured to determine the baseline of these values for each experiment. All values measured were compared with those of the initial control period to ensure the maintained integrity of the placental preparation throughout the course of the perfusion experiment.

The physical integrity of the placental preparation was assessed by monitoring the stability of the fetal perfusion pressure and volume loss from the fetal reservoir; increases or decreases in pressure ( $>10$  mmHg) and volume loss ( $>3$  ml/h) were criteria for rejection of the preparation. After the 1-h control period, the perfusates in both the maternal and fetal circulations were replaced with fresh media, and insulin lispro at a concentration of  $100 \mu\text{U/ml}$  was introduced into the maternal reservoir in four experiments. In four additional perfusion experiments, the concentrations of insulin lispro in the maternal circulation were 200, 580, and  $1,000 \mu\text{U/ml}$ . The maternal circuit was "open" (nonrecirculated) to deliver a con-

**Table 1—Parameters of viability during in vitro perfusion of the human placental cotyledon**

Parameter	Control period	Experimental period	P
O <sub>2</sub> delivery ( $\mu\text{mol} \cdot \text{min}^{-1} \cdot \text{g}^{-1}$ )	$0.02 \pm 0.006$	$0.021 \pm 0.006$	1.00
O <sub>2</sub> transfer ( $\mu\text{mol} \cdot \text{min}^{-1} \cdot \text{g}^{-1}$ )	$1.530 \pm 0.876$	$1.738 \pm 1.001$	0.665
O <sub>2</sub> consumption ( $\mu\text{mol} \cdot \text{ml}^{-1} \cdot \text{g}^{-1}$ )	$0.228 \pm 0.126$	$0.250 \pm 0.060$	0.663
Glucose consumption ( $\mu\text{mol} \cdot \text{min}^{-1} \cdot \text{g}^{-1}$ )	$0.274 \pm 0.193$	$0.285 \pm 0.134$	0.903
Lactate production ( $\mu\text{mol} \cdot \text{min}^{-1} \cdot \text{g}^{-1}$ )	$0.39 \pm 0.21$	$0.18 \pm 0.04$	0.015
Fetal flow rate (ml/min)	$3.44 \pm 0.94$	$3.38 \pm 1.10$	0.908
Fetal inflow pressure (mmHg)	$52 \pm 12.64$	$46.65 \pm 10.98$	0.381
Maternal flow rate (ml/min)	$13.45 \pm 1.11$	$13.18 \pm 1.27$	0.658

Data are means  $\pm$  SD.

stant concentration of drug to the lobule, while the fetal circuit was "closed" (recirculated).

Samples were obtained from the fetal reservoir and the maternal artery and vein every 20 min for the first 2 h and subsequently every 30 min for the last 2 h to measure insulin lispro, antipyrine, lactate, glucose, and hCG concentrations. Antipyrine is a freely diffusible flow-dependent transfer marker to which drug transfer is commonly compared. Oxygen, glucose consumption, and lactate production were measured during the perfusion to confirm that the tissue maintained its ability for energy metabolism. After 4 h of perfusion with insulin lispro, the perfusates in both maternal and fetal reservoirs were replaced again with fresh media and circulations "closed" for a final 1-h control period. All values measured were compared with those of the initial control period to ensure that the integrity of the preparation was maintained during perfusion.

### Sample analysis

Perfusate samples were kept at  $-20^\circ\text{C}$  until analysis. The concentrations of insulin lispro were measured using the insulin lispro competitive-binding radioimmunoassay with overnight equilibrium incubation at room temperature (Linco Research, St. Charles, MO). This assay is highly specific for insulin lispro (100%) and has negligible cross-reactivity with native human insulin or proinsulin ( $<0.5\%$ ). Whereas the limit of quantitation is quoted at  $2.5 \mu\text{U/ml}$  for a  $100\text{-}\mu\text{l}$  sample using the manufacturers' kit, in blank samples of fetal buffer we measured levels of  $5.89 \mu\text{U/ml}$ . Hence, we used this concentration to subtract from all apparent levels measured during the experiment in the fetal circulation. pH and  $\text{pO}_2$

of the perfusate samples were measured by using a blood gas analyzer (Radiometer ABL 330; Radiometer, Copenhagen). The perfusate concentrations of lactate, glucose, and hCG, as well as tissue hCG content, were measured as described previously in our laboratory (15). Antipyrine concentrations were determined spectrophotometrically.

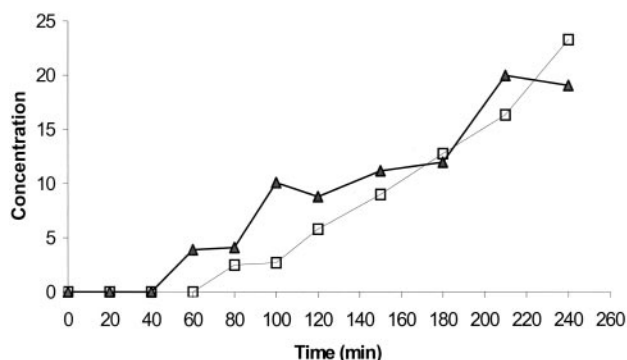
### Statistical analysis

The mean fetal concentration-time relation was plotted graphically. Least square regression analysis was used to study the relation between measured fetal drug concentration and time. The rate of appearance on the fetal circulation was calculated by the mean of the slope of insulin lispro appearance (in microunits) versus time (in minutes) and expressed as microunits per minute per grams of tissue. Comparisons among values were made using the Student's *t* test.

### In vivo studies

In 11 women 31–40 years of age, we measured serum insulin lispro levels 60 min after subcutaneous injection of a single lispro dose of 3–52 units. Of 11 women, 9 were pregnant, 2 had gestational diabetes, 5 had type 1 diabetes, and 3 had type 2 diabetes. One of nine women was in the first trimester, and the others were in the second or third trimester of pregnancy.

**RESULTS**— The mean mass of the perfused cotyledons was  $11.8 \pm 3.4$  g. Placental glucose and oxygen consumption and oxygen delivery and transfer as indicators of metabolic viability of the placental tissue did not change significantly throughout the perfusion experiment (Table 1). The fetal inflow pressures were not different between the control and experimental periods. The overall



**Figure 1**—Time course of insulin lispro appearance in the fetal circulation during 4 h of perfusion with maternal circuit opened and fetal closed. MR, maternal reservoir concentrations.  $\square$ , MR 580  $\mu\text{U/ml}$ ;  $\blacktriangle$  MR 1,000  $\mu\text{U/ml}$ .

rate of lactate production during the control period was  $0.39 \pm 0.21 \mu\text{mol/ml}$ , significantly higher than the rate of lactate production during the experimental period ( $0.18 \pm 0.04 \mu\text{mol/ml}$ ,  $P = 0.015$ ). This is most likely due to the washout of lactate that accumulated in the tissue during the period of hypoxia after delivery.

During the 4-h experimental period with a constant concentration of 100 and 200  $\mu\text{U/ml}$  of insulin lispro on the maternal side of the placenta, insulin lispro was undetectable in the fetal side of the placenta. At higher concentrations of insulin lispro in the maternal circulation (580 and 1,000  $\mu\text{U/ml}$ ), insulin lispro accumulated linearly in the closed fetal circulation (Fig. 1). The accumulation of insulin lispro on the fetal side started after 80 min for the 580- $\mu\text{U/ml}$  concentration and 60 min for the 1,000- $\mu\text{U/ml}$  concentration. The mean calculated rate of insulin lispro transfer was  $0.045 \mu\text{U} \cdot \text{min}^{-1} \cdot \text{g tissue}^{-1}$  at circulating maternal level of 1,000  $\mu\text{U/ml}$  and  $0.019 \mu\text{U} \cdot \text{min}^{-1} \cdot \text{g tissue}^{-1}$  at 580  $\mu\text{U/ml}$  (Table 2).

### In vivo studies

In 11 women treated with insulin lispro, serum levels of insulin lispro ranged from 2 to 576  $\mu\text{U/ml}$  with an apparent linear correlation (Fig. 2). One woman receiving 52 units achieved a level of 576  $\mu\text{U/ml}$ .

**CONCLUSIONS**— The placental perfusion model enables the noninvasive collection of information regarding human placental transfer of molecules (15). Because transfer is measured in intact tissue, the model approximates the in vivo situation more closely than subcellular or cell culture systems. Importantly, it obviates the enormous ethical hurdles of

studying human placental transfer in vivo. At the end of normal pregnancy, postprandial insulin levels are elevated and plasma insulin concentration may reach 100–200  $\mu\text{U/ml}$  (16).

In the current study, we chose an insulin lispro concentration of 100  $\mu\text{U/ml}$  as our baseline level to mimic the peak levels typically measured after administration of  $\sim 13$  units of insulin lispro to healthy volunteers (2). Assuming linear pharmacokinetics, the higher concentrations used in our experiment (200, 580, and 1,000  $\mu\text{U/ml}$ ) would correspond to peak serum insulin levels achieved after subcutaneously administered doses of  $\sim 26$ , 75, and 130 units of insulin lispro, respectively (1). There is an increased need for insulin throughout pregnancy. Women with type 1 diabetes require on average 1.2 units/kg insulin per day; however, the range is broad (16). Women with type 2 diabetes often require larger doses. In one study, women with type 2 diabetes required on average 1.6 units/kg a day or  $\sim 150$  units of insulin per day. Women who are very obese may even

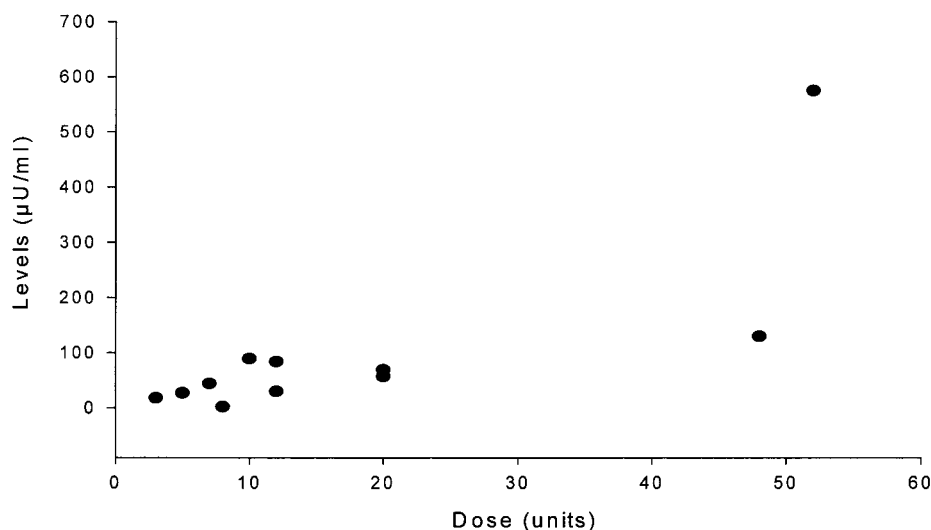
start with 2 units/kg a day, requiring larger doses as the pregnancy progresses (17). Because a typical multiple daily insulin regimen may include  $\sim 40$ – $60\%$  of the total insulin dose used as a short- or rapid-acting insulin analog, a patient with type 1 diabetes in pregnancy may require up to 10–18 units of insulin lispro per meal and a patient with type 2 diabetes in pregnancy potentially requiring up to 20–30 units of insulin lispro per meal. For those women who are very obese and/or very insulin resistant, requirements can go up to 40–60 units of insulin lispro per meal. Among 11 women monitored by us, serum lispro levels were  $<200 \mu\text{U/ml}$  in all those receiving  $<50$  units, whereas higher doses had concentrations associated with placental transfer of the hormone.

The two lower concentrations of insulin lispro used in our experiments (100 and 200  $\mu\text{U/ml}$ ) correspond to peak plasma insulin levels achieved after subcutaneous administration of  $\sim 13$  and 26 units of insulin lispro and suggest that these results are clinically relevant (17). The higher two concentrations (580 and 1,000  $\mu\text{U/ml}$ ) would correspond to subcutaneous doses of insulin lispro of  $\sim 75$  and 130 units, respectively, which are less likely to be utilized clinically.

Our study demonstrated a small placental transfer of insulin lispro at higher concentrations, after at least 60 min of constant concentration in the maternal reservoir. This experimental condition of maintaining high levels in the maternal circulation for several hours does not occur in the clinical setting due to the short elimination half-life of insulin lispro. Hence, even the transfer of insulin lispro shown by us in excessive maternal levels

**Table 2**—Maternal to fetal transport of insulin lispro

Experiment no.	Maternal concentration ( $\mu\text{U/ml}$ )	Lobule weight (g)	Fetal concentration at 4 h ( $\mu\text{U/ml}$ )	Rate of transfer ( $\mu\text{U} \cdot \text{min}^{-1} \cdot \text{g tissue}^{-1}$ )
1	100	16.24	Below limit of quantification	—
2	100	9.7	Below limit of quantification	—
3	100	10.81	Below limit of quantification	—
4	100	16.32	Below limit of quantification	—
5	200	5.15	Below limit of quantification	—
6	580	16.92	23.3	0.019
7	1000	9.43	19.1	0.045
8	1000	8.91	19.9	0.047



**Figure 2**—Dose versus serum levels of lispro. Each data point in the chart represents a single measurement.

with constant concentration on maternal side is not likely to occur in vivo.

Lack of transfer of insulin lispro across the placenta was suggested in a study of insulin lispro in women with gestational diabetes (18). In that study, four women received an intravenous infusion of insulin lispro at a dose of  $0.2 \text{ units} \cdot \text{kg}^{-1} \cdot \text{h}^{-1}$  during labor, and no insulin lispro was detected in the umbilical cord blood of their infants. In our study, maternal insulin lispro concentrations of  $\geq 580 \text{ } \mu\text{U/ml}$  led to a small transfer of insulin lispro across the placenta. To put the results of our study in context, one should compare the placental transfer of insulin lispro to that of human insulin. In a single study by Challier et al. (10), human insulin was shown to appear in the fetal circulation after its introduction in the maternal reservoir. The measured levels by Challier et al., using the same perfusion model that we used, appear to be higher than those measured by us for insulin lispro. In their study, Challier et al. did not address the possibility that the low levels of fetal insulin were due to contamination by the residual blood in the perfused cotyledon. To address this question, we have now measured human insulin levels at the beginning of our perfusion experiment (before insulin lispro was introduced) and thereafter. Insulin levels, measured by a sensitive radioimmunoassay, were below the detection limits. This lends further credibility to the results by Challier et al. showing that human insulin does cross the placenta. Hence, it appears that small amounts of insulin cross the placenta when both

human insulin and insulin lispro are present at clinically high concentrations.

Although the use of third-trimester placenta might be considered a potential limitation to our study, there is evidence to suggest that the qualitative aspects of transfer do not differ between placenta taken at earlier versus later phases of gestation (19).

The placenta of diabetic mothers may exhibit chronic disturbance in intervillous circulation, dilatation of capillaries, and relatively immature villous structure. The impact of these changes on the placental transfer of regular insulin or insulin lispro is unknown.

In summary, when used clinically, insulin lispro is not likely to cross the human placenta. There is a small dose-dependent transfer of insulin lispro across the term human placenta at concentrations  $\geq 580 \text{ } \mu\text{U/ml}$ . These concentrations likely correspond to peak insulin levels achieved with high doses of insulin lispro at  $\sim 75$  units or higher. Only in very rare situations would diabetic women receive doses that would lead to sustained plasma levels sufficiently high ( $580 \text{ } \mu\text{U/ml}$ ) for fetal exposure.

The marginal magnitude of the transfer of insulin lispro in terms of amounts, appears to be less than that shown by others to occur with similar concentrations of human insulin, and hence suggests that insulin lispro does not pose a risk for the fetus.

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