Maternal-fetal transport kinetics of L-Leucine in vitro in gestational diabetic pregnancies

M Nandakumaran, M Al-Shammari, E Al-Saleh

Summary

Objective: Paucity of data relating to transport of amino acids in gestational diabetic pregnancies prompted us to undertake this study. Transport kinetics of a model amino acid, L-leucine was investigated in gestational diabetic pregnancies in vitro, using perfusion of isolated placental lobules.

Methods: Placentae from diabetic and control pregnant women were collected post-partum. Suitable placental lobules were then perfused, using National Culture and Tissue Collection (NCTC) medium, diluted with Earle's buffered salt solution as perfusate. 14C-labelled L-leucine along with tritiated water as reference were injected as a 100 ul bolus into the maternal circulation and serial perfusate samples collected over a 5-minute study period.

Results: In 6 successful perfusions, differential transport rate of L-leucine for 10, 25, 50, 75 and 90% of efflux in the fetal vein averaged 1.17, 1.12, 1.22, 1.20 and 1.17 times respectively that of reference in the diabetic group. In the control group (n = 6), leucine transport indices for the corresponding efflux periods averaged 1.13, 1.15, 1.18, 1.17 and 1.16 times respectively that of the reference marker. Student’s ‘t’ test showed that the difference between the two groups was not statistically significant (p > 0.05) for all the efflux fractions studied. In the diabetic series, leucine transport fraction (TF) averaged 41.2 ± 4.5% of corresponding water TF while in control group, the amino acid TF averaged 46.5 ± 6.5% of water TF. The difference between the two series, however was not statistically significant (p > 0.05). Similarly, kinetic parameters as area under the curve, clearance, elimination constant, time for maximum response, absorption rate, and elimination rate in the diabetic and control groups, were not significantly different (p > 0.05) as well.

Conclusion: Our study seems to indicate that transport kinetics of l-leucine under in vitro conditions, do not differ significantly in placental diabetic women compared to controls.

Key-words: Human Placenta · L-Leucine · In vitro · Maternal-Fetal Exchange · Gestational diabetes.


Resume


Méthodes : Des placenta de femmes enceintes diabétiques et témoins ont été recueillis en post-partum. Des lobules placentaires ont été alors perfusés du milieu NCTC (National Culture and Tissue Collection), dilué par de la solution tamponnée de Earle. La leucine marquée au C14 et de l’eau tritée comme référence ont été injectées en bolus de 100 ul dans la circulation maternelle et des échantillons de perfusat ont été recueillis sur une période de 5 minutes.

Résultats : Dans 6 perfusions successives, le taux de transport différentiel de la L-leucine pour 10, 25, 50, 75 et 90 % de l’efflux dans la veine fœtal était en moyenne 1.17, 1.12, 1.22, 1.20 et 1.17 fois celui de référence dans le groupe diabétique. Dans le groupe contrôle (n = 6), les indices de transport de la leucine pour les périodes correspondantes d’efflux étaient en moyenne 1.13, 1.15, 1.18, 1.17 et 1.16 fois ceux du marqueur de référence. Les différences entre les deux groupes n’étaient pas significatives selon le test t de Student (p > 0,05) pour toutes les fractions d’efflux étudiées. Dans les séries diabétiques, la fraction de transport de la leucine (FT) était en moyenne de 41,2 ± 4,5 % celle de la FT de l’eau correspondante, tandis que dans le groupe témoin, la FT de l’acide aminé était en moyenne de 46,5 ± 6,5 % celle de l’eau. Les différences entre les deux séries n’étaient pas statistiquement significatives (p > 0,05). De même, les paramètres cinétiques tels que l’aire sous la courbe, la clairance, la constante d’élimination, le temps pour la réponse maximale, le taux d’absorption et le taux d’élimination n’étaient pas différents entre le groupe diabétique et le groupe témoin (p > 0,05).

Conclusion : Notre étude semble montrer que les cinétiques du transport de la L-leucine dans des conditions in vitro ne diffèrent pas dans le placenta de femmes ayant un diabète gestationnel par rapport à des femmes témoins.

Mots-clés : Placenta humain · L-Leucine · In vitro · Échanges fréto-maternels · Diabète gestationnel.

Address correspondence and reprint requests to:
M Nandakumaran. Associate Professor, Obstetrics & Gynecology Department, Faculty of Medicine, University of Kuwait, P.O. Box 24923 Safat 13110, Kuwait. moorkath@hsc.kuniv.edu.kw

Received: December 29th, 2003; revised: May 11th, 2004

Diabetes Metab 2004;30:367-74 • © 2004 Masson, all rights reserved
Diabetes mellitus in pregnancy is well recognized to be a serious clinical condition contributing to perinatal morbidity and mortality. Despite being a global problem, data on maternal-fetal exchange of nutrients across the human placenta, in this diseased situation are scanty. There have been some reports in the literature relating to maternal-fetal exchange of nutrients in experimentally induced diabetic rats [1, 2, 3] rabbits [4] and guinea pigs [5]. Studies on placental transport and metabolism of nutrients have been also reported in the non-diabetic pregnant ewe [6], subjected to artificially-induced graded hyperglycemia. Species differences, however do not readily permit comparison as well as extrapolation of animal data to humans. Although the transport behaviour of a model non-metabolisable amino acid, alpha aminoisobutyric acid, belonging to the A-type transport system has been reported in diabetic animals [3, 5] as well as in humans [7], no study, to our knowledge has explored the transport kinetics of l-leucine, a model amino acid belonging to the l-type of transport system [8] in human diabetic pregnancies. We have, hence attempted to investigate maternal-fetal transport kinetics of this essential amino acid in gestational diabetic pregnancies, using in vitro perfusion of human placental lobules. L-leucine has been previously used by several international research groups to study placental permeation characteristics in control pregnancies as well as in disease states [9, 10, 11, 12]. Tritiated water served as the internal reference marker and control placental lobules were perfused for comparison. The method of isolated lobular perfusion has earlier been used successfully by our research group in investigating transport behaviour of variety of nutrients and drugs in normal pregnancies [13, 14, 15], as well as in disease states including diabetes mellitus [7, 16].

Material and methods

We successfully perfused placental lobules from 6 non-insulin-dependent (Type 2) gestational diabetic women (Tab I) and compared the data with successful placental perfusions from 6 normal, uncomplicated pregnancies. All women of diabetic group were clinically diagnosed as gestational diabetics and their blood glucose was controlled within physiological limits by appropriate diet and exercise therapy during course of pregnancy and time of delivery. Women receiving insulin for control of diabetes were not included in the study. Gestational diabetes was diagnosed as per standard WHO criteria. Patients were uniformly screened using GLT (Glucose Loading Test) and GTT (Glucose Tolerance Test) as diagnostic test at the first ante-natal visit and then again at 26-28th weeks of pregnancy Diagnostic GTT test was also performed in patients at 26-28th week of pregnancy, with abnormal obstetric profile such as macrosomia, poly-hydramnios, previous history of diabetes, hypertension, etc. The blood glucose was monitored during every hour of labour of the diabetic patients. Women receiving insulin for control of diabetes were not included in the study. Only those women confirmed as non-diabetic during screening and having no kidney, metabolic or liver disease or hypertension during the duration of pregnancy were considered for inclusion in the control group (Tab I). Further, control women had normal bodyweight, having the body mass index (BMI) range of 18-25 kg/m² and matched with respect to age and gestational age with the study group.

Perfusion Technique

Perfusion of placental lobules was performed according to the technique described previously [17, 13, 14]. Placentae were collected immediately after delivery. Fetal and maternal

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Control group</th>
<th>Diabetic group</th>
<th>Statistical significance (p)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Maternal Age (year)</td>
<td>30.6 ± 4.6</td>
<td>32.3 ± 5.6</td>
<td>0.58</td>
</tr>
<tr>
<td>Prepregnancy Weight (kg)</td>
<td>70.2 ± 2.2</td>
<td>72.1 ± 3.2</td>
<td>0.26</td>
</tr>
<tr>
<td>Weight Gain (kg)</td>
<td>10.2 ± 1.4</td>
<td>10.8 ± 1.2</td>
<td>0.44</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>21.8 ± 0.7</td>
<td>22.1 ± 0.6</td>
<td>0.44</td>
</tr>
<tr>
<td>Previous History of Diabetes, m (%)</td>
<td>2(33%)</td>
<td>2(33%)</td>
<td>1.00</td>
</tr>
<tr>
<td>Gestational Age (weeks)</td>
<td>38.2 ± 0.9</td>
<td>37.1 ± 2.2</td>
<td>0.30</td>
</tr>
<tr>
<td>Weight of Newborn (gm)</td>
<td>3032.3 ± 289.8</td>
<td>3212.6 ± 612.2</td>
<td>0.53</td>
</tr>
<tr>
<td>Weight of Placenta (gm)</td>
<td>546 ± 92.1</td>
<td>592 ± 86.8</td>
<td>0.39</td>
</tr>
<tr>
<td>Blood Glucose at time of Delivery (mmol/L)</td>
<td>4.92 ±0.12</td>
<td>6.20 ±0.14</td>
<td>0.008 **</td>
</tr>
</tbody>
</table>

Values represent means ± s.e.m of 6 patients in each group. Statistical significance of data was assessed by Student’s t-test, Fischer Exact test or Welch’s test; wherever appropriate. ** Statistically Significant (p < 0.01).
circulations of suitable lobules were then cannulated and perfused using National Culture and Tissue Collection (NCTC) medium (Sigma Chem. Co., St. Louis., USA) diluted with buffered, oxygenated Earle’s salt solution [18, 19] as the perfusate. Circulation of the perfusate was effected by Harvard Digital Pump. Perfusate flow rates in both the circuits were measured by flow-metres (BROOKS R215A) and perfusion pressures were monitored by mercury manometers.

After an initial wash-out phase of 10 minutes, 1 uCi of tritiated water (Specific activity: 5 mCi/mmole; Amersham, UK) and 0.5 uCi of [1-14C]labelled L-Leucine (Specific activity: 54 mCi/mmole; Amersham, UK) were injected as a 100 ul bolus into the maternal circulation, at a site close to the insertion of microcannulae in the basal plate. After a period of 1 minute, serial perfuse samples were then collected from the fetal venous outflow for every 15 seconds for a period of 5 minutes. The time lag of 1 minute was determined in previous control experiments, by injecting a bolus of 100 ul Indian ink into the maternal circulation and by noting the time interval required for the dye to appear first on the basal plate. The site, dose and duration of bolus injection were kept identical throughout the duration of the study. Viability of perfusions was assessed by verifying absence of perfusate flow rate mismatch between fetal arterio-venous flows and by determining the O2 consumption of the tissue [20] during the period of perfusion. Radioactivity (dpm/ml) contained in the perfusate samples as well as in the injectate was determined by liquid scintillation counting, using pre-adjusted windows and external standardization, to minimize radioactivity cross-over. Necessary calculations were done to take into account quenching in coloured samples.

Fetal perfusion rate (QF) in diabetic series averaged 4.6 ±0.2 ml/min while in the control perfusions, QF averaged 4.4 ±0.05 ml/min. The maternal perfusion flow rates (QM) in diabetic and control groups averaged 11.6 ±0.6 ml/min, 11.2 ±0.04 ml/min respectively. The fetal-maternal flow ratio (QF/QM) averaged 0.40 ±0.02 in the diabetic series, while in the controls, the flow ratio averaged 0.40 ±0.01. Pressure in maternal arterial circuit averaged 52.0 ±2.2 mm Hg and 54.2 ±3.2 mm Hg in the control and diabetic series respectively. The pressure in the fetal arterial circuit averaged 20.2 ±3.6 and 23.2 ±2.8 mm Hg respectively in control and diabetic series respectively. Cotyledon weights averaged 38.0 ±6.3 g and 29.8 ±3.2 g in the diabetic and control groups respectively. Oxygen consumption of perfused lobules in control series averaged 0.012 ±0.006 umol/min/g while in study series, the oxygen consumption averaged 0.014 ±0.008 umol/min/g.

Transport Parameters

1) Maternal-fetal transport of l-leucine and tritiated water were expressed as differential transport rate (TR) expressed as time in minutes for a given fraction to be transported across to the fetal vein [21, 19].

Efflux Fraction = \frac{\text{\( ^{3} \text{H} \) or \( ^{14} \text{C} \) dpm in fetal venous sample}}{\text{Total \( ^{3} \text{H} \) or \( ^{14} \text{C} \) dpm in fetal vein for a period of 5 minutes}}

From the curve obtained by plotting various cumulative efflux fractions as a function of perfusion time, time in minutes for efflux of 10, 25, 50, 75 & 90% of total venous efflux were then computed. Control studies had established that in a given lobule, the above-mentioned efflux times for study substances varied little with increasing or decreasing doses, for a given set of feto-maternal flow rates. Leucine transport rates in both the series were also expressed as transport rate indices, as ratio of corresponding reference transport rates.

2) Transport fraction (TF) of substances studied [21, 19] were calculated thus:

\[
TF = \frac{\text{Total \( ^{3} \text{H} \) or \( ^{14} \text{C} \) dpm in fetal venous efflux for a period of 5 minutes}}{\text{Total \( ^{3} \text{H} \) or \( ^{14} \text{C} \) dpm in the injected bolus}}
\]

Calculation of the ratio relating to TF of test substance studied, to that of the reference substance will permit the researcher to assess transport index (TI) of the former, independent of factors as minor variations in flow rate, shunts, changes in membrane surface area, etc.

3) Areas under the curve (AUC) of study and reference substances were determined by trapezoid rule, using the following formula and assuming a two-compartment model [21, 22, 23] where C = concentration of the substance in the sample; t = time in seconds of sample collection, n = number of experimental points; C(n) = last experimental point or measurement; Kel = elimination constant.

\[
\text{AUC} = \frac{\sum_{i=1}^{n} [C(i+1)+C(i)] x [t(i+1) - t(i)]}{2} \times C(n) + C(1)
\]

Parameters as clearance, Tmax, Kel, absorption rate and elimination rate were calculated using a computer programme based on IMSL Fortran Subroutine software, specially adapted for statistical applications. The kinetic parameters of the amino acid were also expressed as ratio or index of corresponding reference marker value, to take into account factors as minor variations in flow rate, possible changes in membrane surface area, shunts, etc as described earlier in the text.

4) To assess the active or passive nature of leucine transport, another parameter [16, 19] was also employed. The leucine label counts in the different perfusate samples in the fetal vein were expressed as a fraction of injected maternal bolus and the ratios plotted as a function of perfusion time. It was premised that total radioactivity in injected amino acid bolus being identical in the two series, any alteration in slope in leucine TF ratio in fetal venous effluent as
a function of perfusion time will be a good indicator of membrane transport behaviour in the two series. Transport rate slopes between the study and control groups were compared using analysis of co-variance, to assess the difference, if any in transport function of the substance investigated.

Data Analysis

Statistical analysis of data was done by means of Analysis of variance (ANOVA), analysis of co-variance (ANCOVA) and paired or unpaired Student’s t-test were used, wherever appropriate (Graphpad Instat Program using Graphpad Software V2-05a). The results are expressed as means ± s.e.m. A probability value < 0.05 was considered to be statistically significant.

Results

Characteristics of control and diabetic patients are shown in Table I. Student’s t-test showed no significant difference (p > 0.05) between age, prepregnancy weight, weight gain, BMI, previous history of diabetes, gestational age, weight of newborn and weight of placenta between study and control groups. However, blood glucose at delivery was significantly higher (p < 0.05) in the study group compared to that of control. Maternal and fetal perfusion rates and pressures as well as oxygen consumption values in the study and control groups did not differ significantly (Student’s t-test; p > 0.05). Differential transport rates of l-leucine for 10, 25, 50, 75 and 90% efflux fractions in the fetal vein averaged 0.68 ± 0.03, 1.10 ± 0.06, 1.90 ± 0.07, 3.31 ± 0.26 and 4.10 ± 0.25 minutes respectively in the diabetic series while in controls the transport rates for the above efflux fractions averaged 0.57 ± 0.08, 0.99 ± 0.06, 1.76 ± 0.06, 2.82 ± 0.24 and 3.98 ± 0.27 minutes respectively. The corresponding water transport rates for the different efflux fractions averaged 0.48 ± 0.09, 0.82 ± 0.11, 1.45 ± 0.06, 2.56 ± 0.09 and 3.60 ± 0.08 minutes respectively. No significant difference between leucine and water transport rates could be shown in the control and study group (Student’s t-test; p > 0.05). Leucine transport rate indices, expressed as ratio of corresponding water transport rates in diabetic series averaged 1.15, 1.17, 1.22, 1.14 and 1.12 for 10, 25, 50, 75 and 90% efflux fractions (Tab III) while in the control placentae, the indices for corresponding fractions averaged 1.18, 1.21, 1.21, 1.10 and 1.10 times respectively.

Table II
Differential transport rates of tritiated water in control & diabetic pregnancies.

<table>
<thead>
<tr>
<th>Perfusion</th>
<th>TR 10</th>
<th>TR 25</th>
<th>TR 50</th>
<th>TR 75</th>
<th>TR 90</th>
<th>Flow ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control Perfusions</td>
<td>0.48 ± 0.09</td>
<td>0.82 ± 0.11</td>
<td>1.45 ± 0.06</td>
<td>2.56 ± 0.09</td>
<td>3.60 ± 0.08</td>
<td>0.40 ± 0.01</td>
</tr>
<tr>
<td>Diabetic Perfusions</td>
<td>0.58 ± 0.11</td>
<td>0.94 ± 0.09</td>
<td>1.55 ± 0.18</td>
<td>2.67 ± 0.06</td>
<td>3.71 ± 0.09</td>
<td>0.40 ± 0.02</td>
</tr>
<tr>
<td>Statistical Significance (p)</td>
<td>0.12</td>
<td>0.07</td>
<td>0.08</td>
<td>0.07</td>
<td>0.12</td>
<td>1.00</td>
</tr>
</tbody>
</table>

Means ± s.e.m of 6 perfusions in each series. TR = Transport Rate for respective efflux fraction. Statistical Significance (p) was assessed by Student’s t-test.

Table III
Leucine Transport Rate Indices (TR I) In control and diabetic pregnancies.

<table>
<thead>
<tr>
<th>Perfusion</th>
<th>TR 10</th>
<th>TR 25</th>
<th>TR 50</th>
<th>TR 75</th>
<th>TR 90</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control perfusions</td>
<td>1.18 ± 0.04</td>
<td>1.21 ± 0.03</td>
<td>1.21 ± 0.04</td>
<td>1.10 ± 0.05</td>
<td>1.10 ± 0.05</td>
</tr>
<tr>
<td>Diabetic perfusions</td>
<td>1.15 ± 0.03</td>
<td>1.17 ± 0.04</td>
<td>1.22 ± 0.03</td>
<td>1.14 ± 0.06</td>
<td>1.12 ± 0.05</td>
</tr>
<tr>
<td>Statistical significance (p)</td>
<td>0.17</td>
<td>0.08</td>
<td>0.63</td>
<td>0.24</td>
<td>0.50</td>
</tr>
</tbody>
</table>

Values represent means ± s.e.m of 6 perfusions. TR = Transport Rate; TRI = Leucine Transport Rate/Water Transport rate for respective efflux fractions. Statistical significance of data was assessed by Students’ t-test.
Student’s ‘t’ test showed that the transport indices of the amino acid in the two series for corresponding efflux fractions were not significantly different (p > 0.05). Leucine transport fraction, expressed as ratio of corresponding water TF averaged 41.2 ± 4.2% in diabetic series (Fig 1) while in the control group, leucine TF amounted to 46.5 ± 6.5% of tritiated water TF. Student’s ‘t’ test showed that the difference in leucine TF indices in the two groups was not significantly different (p > 0.05). Similarly, no significant difference could be shown (Student’s t-test; p > 0.05) when leucine TR50 values were expressed as ratio or index of corresponding reference marker values (Fig 1) as well.

Results on water and leucine transport kinetics in diabetic and in control series are detailed in Tables IV and V respectively. AUC, Kel, Clearance, Tmax, absorption rate and elimination rate of tritiated water averaged 867,458 dpm/hr/g, 11.50, 8.3 ml/min, 138.6 seconds, 1231 dpm, and 2012 dpm respectively in the former while in the latter the corresponding values averaged 912,320 dpm/hr/g, 9.97, 22.1 ml/min, 132.5 seconds, 1153 dpm and 1842 dpm respectively. Analysis of variance (ANOVA) showed that water and leucine kinetic parameters differed significantly (p < 0.05) in both control as well as study groups. All the same, when water and leucine kinetic parameters were compared with corresponding parameters in the control and study series by Student’s t-test, no statistical significance (p > 0.05) could be shown between the two groups. Similarly, when the leucine kinetics parameters were expressed as ratio or index of corresponding reference values (Tab V), no significant difference (p > 0.05) could be shown between the two groups as well. Absorption rate indices of the amino acid in the diabetic and control groups averaged 36 ±3% and 43 ±9% respectively of reference value while the elimination rate indices in the two groups averaged 34 ±6% and 42 ±6% respectively. Absorption: elimination rate index averaged 1.03 ±0.03 in the diabetic series while in controls, ...

---

**Table IV**
Pharmacokinetic transport parameters of tritiated water in control & diabetic pregnancies.

<table>
<thead>
<tr>
<th>Perfusions</th>
<th>AUC (dpm/hr/gm)</th>
<th>Clearance (ml/min)</th>
<th>Kel</th>
<th>Tmax (sec)</th>
<th>Absorption Rate (dpm)</th>
<th>Elimination Rate (dpm)</th>
<th>Cytodoned Weight (gm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control Series</td>
<td>912320 ± 48345</td>
<td>22.1 ± 3.6</td>
<td>9.97 ± 1.50</td>
<td>132.5 ± 4.8</td>
<td>1153 ± 119.6</td>
<td>1842 ± 242.6</td>
<td>29.8 ± 3.2</td>
</tr>
<tr>
<td>Diabetic Series</td>
<td>867458 ± 66784</td>
<td>8.3 ± 6.90</td>
<td>11.50 ± 1.20</td>
<td>138.6 ± 6.4</td>
<td>1231 ± 143.2</td>
<td>2012 ± 286.8</td>
<td>38.0 ± 6.3</td>
</tr>
<tr>
<td>Statistical Significance (p)</td>
<td>0.21</td>
<td>0.08</td>
<td>0.09</td>
<td>0.12</td>
<td>0.33</td>
<td>0.29</td>
<td>0.09</td>
</tr>
</tbody>
</table>

Means ± s.e.m of 6 perfusions in each series. AUC = area under the curve; Kel = Elimination constant; Tmax = Time for maximum response; dpm = disintegrations per minute. Statistical Significance of data was assessed by Student’s t-test or Analysis of variance, wherever appropriate.

**Table V**
Pharmacokinetic Transport Indices (TI) of Leucine in control & diabetic pregnancies.

<table>
<thead>
<tr>
<th>Perfusions</th>
<th>AUC</th>
<th>Clearance</th>
<th>Kel</th>
<th>Tmax</th>
<th>Absorption Rate</th>
<th>Elimination Rate</th>
<th>Cytodoned Weight (gm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control Series</td>
<td>0.36 ± 0.07</td>
<td>2.21 ± 0.60</td>
<td>0.97 ± 0.50</td>
<td>1.15 ± 0.04</td>
<td>0.43 ± 0.09</td>
<td>0.42 ± 0.06</td>
<td>29.8 ± 3.2</td>
</tr>
<tr>
<td>Diabetic Series</td>
<td>0.28 ± 0.06</td>
<td>3.29 ± 0.90</td>
<td>2.50 ± 1.20</td>
<td>1.24 ± 0.06</td>
<td>0.36 ± 0.06</td>
<td>0.34 ± 0.06</td>
<td>38.0 ± 6.3</td>
</tr>
<tr>
<td>Statistical Significance (p)</td>
<td>0.06</td>
<td>0.07</td>
<td>0.09</td>
<td>0.10</td>
<td>0.12</td>
<td>0.08</td>
<td>0.09</td>
</tr>
</tbody>
</table>

Means ± s.e.m of 6 perfusions in each series. TI = Leucine Value/Reference Marker Value. AUC = area under the curve; Kel = elimination constant; Tmax = time for maximum response. Statistical Significance of data was assessed by Student’s t-test or Analysis of variance, wherever appropriate.
The index averaged 1.02 ± 0.02. Student’s ‘t’ test showed that the above indices were not significantly different (p > 0.05) in the study and control groups. Figure 2 shows cumulative transport fraction curves of l-leucine in the fetal vein, expressed as ratio of injected amino acid bolus load in control and diabetic series. Analysis of co-variance (ANCOVA) showed that the slopes of the two curves were not significantly different (p > 0.05).

Discussion

This study, to our knowledge is the first to have investigated l-leucine transport kinetics from maternal to fetal circulation in vitro in gestational diabetic pregnancies. As explained elsewhere [9, 13], the in vitro perfusion method has provided us with the unique opportunity of exploring maternal - fetal transport under controlled experimental conditions in humans, without recourse to invasive in vivo experimentation.

Results in control placentae demonstrated that l-leucine is transferred freely across the placental membrane, with transport fraction representing 46.5% of tritiated water TF. These findings are comparable with those of Schneider et al. [9, 18], reported in the perfused human placental lobule, in steady-state perfusion conditions and agree with the findings of Cetin et al. [10] in humans in vivo. Leucine TF in diabetic placentae averaged 41.2% of corresponding water TF. Though the amino acid transport appeared to be less efficient than that of controls, no statistical significance could be demonstrated between the two, due to inter-experimental variability. A similar phenomenon was observed in the case of amino acid AUC as well as absorption rate data as well. Further, though leucine transport rate curve was apparently lower than that of control group, no significant difference could be shown between the two groups. Similarly, no statistical significance between TR50 indices in control and diabetic placentae could be shown for the amino acid between control and study groups indicating possibility of unaltered l-leucine transport in the disease state. Further analysis of transport kinetic data led to similar conclusions. Though leucine absorption rates and elimination rates were lower in study group than in controls, no statistical significance could be shown between the two groups. Similarly, the absorption rate: elimination rate ratios of the amino acid were apparently lower in the diabetic group than that of controls, but no statistical significance could be demonstrated between the two. These findings are in agreement with the observations of Kuruvilla et al. [12] in human diabetic pregnancies. The authors observed that activity of leucine transporter system (System L) did not differ significantly between control and study groups with or without fetal macrosomia. However, contrary to report of Kuruvilla et al., Jansson et al. [24] have reported accelerated l-leucine uptake of placental vesicles from gestational diabetic women with macrosomic babies. The reasons for having contrasting findings from two research groups utilizing comparable research methodology are unclear and deserves further detailed study. Since there was no fetal macrosomia in our control and study groups, our results are not comparable with those of the Swedish research group [24]. We are unable to speculate whether the leucine transport kinetics would have been different in placentae of gestational diabetic mothers with macrosomic babies. Interestingly, unpublished research from our laboratory seems to indicate that hyperglycemia per se has no significant effect on transport kinetics of l-leucine in the perfused human placental lobule of normal pregnancies in vitro.

The role of placentally transferred amino acids in genesis of fetal macrosomia in some diabetic pregnancies remains unclear. Although such a possibility has been implied by some authors [25], reports of lower amino acid concentrations in infants of diabetic mothers [26] and unchanged or lower maternal amino acid levels reported in experimentally induced diabetic rats [1, 27] contradict such a possibility. The finding of unaltered l-leucine transport kinetics in this study tend to indicate that hyperglycemia per se may be the most important factor for fetal macrosomia [28] in some diabetic pregnancies. Further studies, however are needed before definitive conclusions can be drawn.

Though it is generally believed that the placental tissue per se is insensitive to insulin control of glucose metabolism, the influence of the hormone in determining glucose utilization of maternal and fetal tissue and the resultant effect on amino acid utilization in patients receiving insulin for control of hyperglycemia cannot be overlooked. Previous
research in our laboratory [29] had established that insulin does stimulate maternal-fetal transport of another model amino acid, alpha-aminooisobutyric acid (AIB) in vitro. Although placental transport of l-leucine is not mediated through the A type transporter system [8], possibility of a similar effect of insulin on its transport in patients requiring insulin therapy cannot be excluded.

It needs to be emphasized here that being an in vitro, short term study our results on leucine transport kinetics in gestational diabetic pregnancies cannot be extrapolated directly to in vivo situation in humans. The possibility of interplay of several maternal as well as fetal factors in vivo make direct comparison or extrapolation of our data to the former situation difficult and untenable. Further studies involving a larger sample size are currently underway in our laboratory to validate findings of this pilot study.

The fetal and maternal perfusate flows were maintained in the physiological range in this study and such an ideal situation may not always be present in diabetic pregnancies. In fact, many investigators have reported villous abnormalities [30], uneven intervillous perfusion [31] and greatly reduced uteroplacental blood flow [31] in diabetic women, factors that could severely compromise placental perfusion and limit the exposure of available substrate load at the membrane interface. Results from our laboratory [32] have indicated that hyperglycemia within pathological disease limits does modify transport kinetics of the model amino acid, AIB. Although leucine transport is effected principally through a different transporter system than that of AIB [8], unpublished results from our laboratory have not shown any such restraining effect of hyperglycemia on transport of l-leucine in placentae from normal women. Presently, studies are underway in our laboratory exploring effects of hyperglycemia on maternal-fetal transport of nutrient analogues in control and diabetic placentae, in varying experimental conditions.

Acknowledgements – The authors wish to thank Mrs. Nirmala Narayanan, Mrs. Susan George and Mrs Ajitha Suresh for their excellent technical help. The work was supported by Kuwait University Research Grants No: MOO26 and MOO32.

References