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Increase in fluidity of human placental syncytiotrophoblastic brush-border membrane with advancement of gestational age: a fluorescence polarization study

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Abstract

The syncytiotrophoblastic brush-border membrane (BBM) of human placenta plays a vital role in the exchange of metabolites between mother and developing fetus. The fluidity of this membrane renders its selective permeable character. To meet the changing needs of the growing fetus with the progress of gestational age changes in the composition and properties of BBM occur. In the present study, decrease in cholesterol/phospholipid ratio of BBM indicated an increase in fluidity with advancement of gestational age. Measurement of the steady state fluorescence anisotropy of labeled liposomes prepared from lipid extract of BBM as well as that of labeled BBM vesicles prepared from native BBM shows decrease in restriction of the bound dye molecule with increased gestational age. Decrease in transition temperature of BBM and enhanced glucose transport through it have been observed with advancement of pregnancy.

Keywords: Placenta; Brush-border membrane; Liposome; Fluidity; Transition temperature; Glucose transport

1. Introduction

The selective nature of biological membranes depends on their lipid composition, which satisfy demands related to membrane structure, fluidity and permeability, as well as protein association and function. The fluidity of biological membranes has been found to be a major determinant in regulating transport processes [1].

A prominent function of human placental epithelia is to maintain adequate delivery of maternal blood-borne nutrients to the developing fetus. The syncytiotrophoblast is well suited to the purpose of transcellular transfer as its apical or maternal surface exists in the form of a brush-border [2]. With the progress of gestational age the metabolic need of the fetus changes, hence the brush-border membrane (BBM) has to modify its functional role and structural composition. The BBM of human placental syncytiotrophoblast can be considered as a functional organelle to investigate the contribution of its different components to absorption and transport, as well as the influence of specific organization on function and control in membrane system.

While working with mid-gestational (14–18 weeks) and term ($\geq$ 35 weeks) placental BBM we found distinct differences in their cholesterol (Ch) and phospholipid (Pl) contents and decrease in Ch/Pl ratio, which indicated a probable change in the fluidity of BBM at different gestational ages [3]. In the present study, we have investigated the changes in anisotropy and transition temperature (T_m) of 1,6-diphenyl-1,3,5-hexatriene (DPH) labeled liposomes prepared from human placental syncytiotrophoblastic BBM lipids as well as those of native BBM, using fluorescence polarization technique to monitor fluidity changes. The effect of changes in fluidity on transport of glucose across placental BBM has also been studied by preparing BBM vesicles (BBMV) from developing and term placentas.

2. Materials and methods

2.1. Materials

1,6-Diphenyl-1,3,5-hexatriene (DPH) and tris-(hydroxymethyl)aminomethane (Tris) were purchased from Sigma,

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St. Louis, MO, USA. D-[^{14}C]Glucose was a gift from Dr. K.D. Mukherjee, Federal Centre of Lipid Research, Muenster, Germany. *N*-2-Hydroxyethylpiperazine-*N'*-2-ethanesulfonic acid (Hepes buffer) and all other reagents were of analytical grade and were purchased locally.

2.2. Clinical materials

Human placentas of gestational ages between 6 to 24 weeks were collected from patients undergoing legal abortion via hysterotomy from the Department of Obstetrics and Gynaecology, National Medical College and Hospital, Calcutta. Term placentas were collected at the time of parturition or via caesarean section from different hospitals in Calcutta. Tissues were collected within 15 min of operation/delivery and kept on ice. Gestational ages were calculated from the period of amenorrhea and by crown-rump length of the fetus [4].

2.3. Isolation of brush-border membrane (BBM)

The BBMs were isolated from developing and term placentas primarily following the method of Balkovetz et al. [5]. The placentas were processed immediately after collection. Removing the maternal decidua using sharp blade, the placental villous tissues (the central part of the placenta) were removed from the chorionic plate. 20 g of tissue was taken and cut into small pieces so as to expose the syncytiotrophoblastic brush-border membrane (BBM). All the subsequent steps were performed at 4° C. The tissues were washed repeatedly with 10 mM Hepes/Tris buffer (pH 7.0) containing 300 mM mannitol to remove blood. The suspension of the tissue in the same buffer was agitated on a magnetic stirrer to loosen the BBM from the syncytiotrophoblast, filtered through cheese cloth and centrifuged at 60 000 × *g* for 30 min. The pellets, representing crude BBM were separated from BBM by treating the suspension with $MgCl_2$ followed by centrifugation at 3000 × *g* for 15 min. The supernatant was spun at 60 000 × *g* for 30 min and pellets were suspended in 3 ml of the above buffer and passed through a 26 gauge needle. The purity of BBM was monitored by assaying the enzyme alkaline phosphatase which is a marker enzyme for the BBM.

2.4. Preparation of brush-border membrane vesicles (BBMV)

BBMVs were prepared from developing and term human placentas by divalent cation aggregation and differential centrifugation following primarily the method of Grassl [2]. The villous tissue of placenta obtained within 15 min of operation was quickly dissected and minced into small pieces at 4° C. The tissue fragments were rinsed well in 10 mM Hepes/Tris buffer, pH 7.0, containing 300 mM mannitol and gently stirred for 30 min. The suspension was filtered through cotton gauze and phenyl methane sulfonyl fluoride was added to a final concentration of 0.2 mM. The filtrate was centrifuged at 5900 × *g* for 15 min, the resulting supernatant was spun at 33 000 × *g* for 40 min and $MgCl_2$ was added to a final concentration of 12 mM. After 30 min of incubation the membrane suspension was centrifuged at 2300 × *g* for 15 min to pellet the Mg^{2+} induced membrane aggregates. The supernatant was spun again for 40 min at 33 000 × *g* and the resulting pellet (BBMV) was washed twice in buffer designated for different experiments. The purity of BBMV was routinely examined by assaying alkaline phosphatase activity.

2.5. Estimation of lipids

BBM lipid was extracted and purified by the method of Folch et al. [6] using chloroform/methanol mixture (2:1, v/v). Total lipid was estimated gravimetrically.

Cholesterol was estimated chemically using acetic acid, $FeCl_3$ solution and concentrated H_2SO_4 [7], as Higgins suggested it to be better than enzymic methods in case of membranes [8].

Phospholipids were assayed according to the method of Fiske and SubbaRow, using 70% perchloric acid, conc. H_2SO_4 and ammonium molybdate [9].

2.6. Estimation of protein

Protein was estimated according to the method of Lowry et al. [10] using bovine serum albumin (BSA) as standard.

2.7. Preparation of DPH-labeled BBMV

BBMVs prepared from placentas of different gestational ages (0.8 mg protein/ml) were incubated with 3.3 μM DPH in 10 mM Tris-HCl buffer (pH 7.4) for 5 min at 25° C. After termination of the reaction by dilution with the same buffer and centrifugation at 25 000 × *g* for 20 min, the pellets obtained were washed twice and resuspended in the same buffer.

2.8. Preparation of DPH-labeled liposomes

The lipids from human placental BBM of different gestational ages and from term were extracted in 10 volumes of chloroform/methanol (2:1, v/v) mixtures and the resultant chloroform phase was evaporated to dryness with a stream of nitrogen gas followed by vacuum. Appropriate amounts of 10 mM Tris-HCl buffer (pH 7.4) and 0.33 μM DPH (as a final concentration) were then added and the dispersion was sonicated with an ultrasonic disruptor, Sorius and Materials-Vibra Cell, until it became clear [11]. Standard conditions were established so that 1 ml of liposome containing 25 μg BBM lipid was prepared.

Table 1
Gestational variation in some biochemical components of developing human placental brush border membrane

Gestational ages (weeks)	Total protein (in mg)	Total lipid (in mg)	Lipid/Protein	Cholesterol (Ch) concentration (in mg/mg of protein)	Phospholipid (Pl) concentration (in mg/mg of protein)	Ch/Pl
Early (6–12)	4.32 ± 0.06	8.88 ± 0.15	2.05	0.33 ± 0.01	0.74 ± 0.04	0.45
Mid (12–24)	6.03 ± 0.09	12.42 ± 0.21	2.06	0.25 ± 0.02	0.93 ± 0.02	0.27
Term (≥ 35)	9.24 ± 0.03	19.38 ± 0.12	2.09	0.17 ± 0.02	1.13 ± 0.04	0.15

Values are expressed as means ± S.E. of five experiments, each with a different placenta.

2.9. Fluorescence measurement

Fluorescence measurements were carried out using a Perkin Elmer MPF-44B Fluorescence Spectrophotometer at different temperatures. The excitation and emission wavelengths used in the DPH fluorescence measurements were 360 nm and 430 nm, respectively. The steady state fluorescence polarization of DPH-labeled membranes and liposomes was expressed as the fluorescence anisotropy, r, using the equation: $r = (I_V \cdot G - I_H)/(I_V \cdot G + 2I_H)$ according to North et al. [12]. I_V and I_H represent the fluorescence intensities of the vertically and horizontally polarized light of the emission with vertically polarized excitation, respectively. G is a correction factor defined as I_H/I_V for DPH freely tumbling in ethanol and excited by vertically polarized light [12]. The anisotropy parameter $((r_0/r) - 1)^{-1}$ was plotted against temperature to determine the transition temperature at different gestational ages. The value of r_0, the maximal limiting anisotropy, used in the calculation was 0.362 [13]. Ohyashiki et al. suggested that the error due to light scattering of the sample emission could be entirely prevented using a cutoff filter [14]. Thus, in the fluorescence anisotropy measurement of DPH-labeled liposomes and BBMVs a 390 nm cutoff filter was used.

2.10. Sodium dodecyl sulfate (SDS)-polyacrylamide gel electrophoresis (PAGE)

SDS-PAGE was conducted in a 7.5% separating and a 3.5% stacking gel system in presence of 0.1% SDS with early (E), mid (M) gestational and term (T) placental BBM. Protein loaded per lane was 100 μg for lanes E,M,T and 150 μg for E′,M′,T′. A constant current of 30 mA per slab was applied and electrophoresis was carried out at 15° C. Protein fractions in a gel were stained using Coomassie brilliant blue [14].

2.11. Glucose uptake studies

Uptake of ^{14}C-labeled D-glucose into BBMVs was measured by Millipore filtration technique following the method of Bissonnette et al. [15] with some modification. Experiment was initiated by addition of 50 μl BBMV to 200 μl of incubation medium which was consisted of buffer A (1 mM Tris-Hepes buffer, pH 7.4, containing 300 mM D-mannitol and 1 mM $MgCl_2$) and 1 mM D-glucose (containing 15 μCi/ml D-[^{14}C]glucose). However, for dose-response experiment, different concentrations of D-glucose were used. Uptake was stopped by withdrawing 20 μl mixture at different time intervals and diluting it in 1 ml ice-cold stop solution (0.9% NaCl solution supplemented with 0.5 mM phenolphthalein). The vesicles were rapidly separated by suction through 0.45 μm Millipore filter papers. The filter papers were washed with 10 ml stop solution and placed in 15 ml liquid scintillation fluid. The radioactive count was taken in a 1209 Rack Beta Liquid Scintillation Counter (LKB Wallac).

2.12. Statistical analysis

A statistical analysis was carried out by computing 't' statistics using the values of glucose uptake at different gestational ages.

Table 2
Fluorescence anisotropy (r) study of DPH-labeled liposomes prepared from BBM and DPH-labeled native BBM of different gestational ages

Gestational age (in weeks)	r of DPH-labeled liposomes at different temperatures						r of DPH-labeled native BBM at 37° C	Transition temperature (in ° C)
	20° C	25° C	30° C	35° C	37° C	40° C		
6– 9	0.254 ± 0.002	0.250 ± 0.002	0.246 ± 0.003	0.244 ± 0.002	0.243 ± 0.001	0.242 ± 0.001	0.252 ± 0.003	28.25
9–12	0.244 ± 0.002	0.236 ± 0.001	0.230 ± 0.003	0.229 ± 0.001	0.227 ± 0.002	0.226 ± 0.002	0.235 ± 0.002	28.00
12–16	0.231 ± 0.001	0.228 ± 0.002	0.224 ± 0.001	0.221 ± 0.002	0.220 ± 0.002	0.218 ± 0.001	0.226 ± 0.001	27.50
16–21	0.224 ± 0.002	0.219 ± 0.001	0.215 ± 0.002	0.212 ± 0.003	0.210 ± 0.003	0.209 ± 0.002	0.218 ± 0.004	27.25
21–24	0.222 ± 0.001	0.216 ± 0.002	0.212 ± 0.001	0.209 ± 0.001	0.208 ± 0.001	0.207 ± 0.001	0.215 ± 0.003	27.00
≥ 35	0.194 ± 0.002	0.189 ± 0.001	0.185 ± 0.002	0.182 ± 0.001	0.181 ± 0.001	0.179 ± 0.002	0.191 ± 0.001	26.00

Values are expressed as means ± S.E. for triplicate determinations in each case.

3. Results

Table 1 shows the variation of some of the basic components of the BBM. The concentration of protein has the lowest value at early gestational age (upto 12th week) and increases steadily till term. The total lipid content also shows the same trend but remaining higher than that of protein throughout pregnancy. The ratio of lipid and protein remains almost constant. Ch decreases with gestational age while Pl, the major component of BBM, shows increasing trend. There is a steady decrease in Ch/Pl ratio with the progress of pregnancy.

Table 2 shows the fluorescence anisotropy (r) of DPH-labeled native BBMVs at 37° C and that of DPH-labeled liposomes at different temperatures. It has been observed that with the progress of gestational age, at any specific temperature, r decreases. On the other hand, for any gestational age, r decreases with increasing temperature. In case of DPH-labeled BBMVs, prepared from native BBMs, r at 37° C shows a steady decrease with increasing gestational age. Since, $r \propto 1/\text{fluidity}$, it may be concluded that with the increasing temperature the fluidity of placental syncytiotrophoblastic BBM increases with the progress of pregnancy.

Anisotropy parameters $((r_0/r)-1)^{-1}$ were calculated from r at different temperatures and plotted against temperature. As all these plots were of same nature, the graphs for only three gestational age groups are presented (Fig. 1). In all these plots a breakpoint is observed, which is the transition temperature (T_m), the temperature at which membrane lipids undergo phase transition, i.e., from gel-crystalline phase to liquid-crystalline phase. T_m for different gestational ages was determined from plots and are presented in Table 2. It may be noted that with the progress of gestational age T_m decreases and is the highest

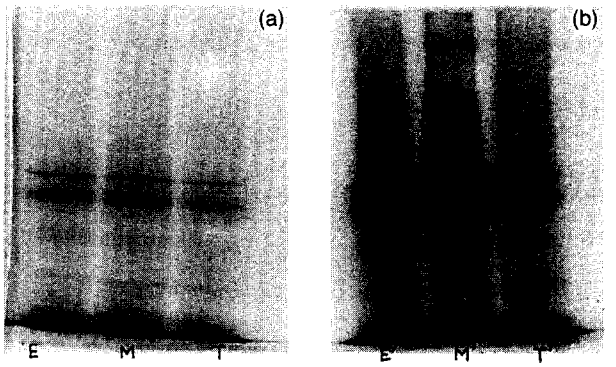

Fig. 2. 7.5% SDS-polyacrylamide gel electrophoresis of early (E and E'), mid (M and M') and term (T and T') placental BBM proteins. Electrophoresis was carried out with (a) 100 μg and (b) 150 μg protein loaded per lane.

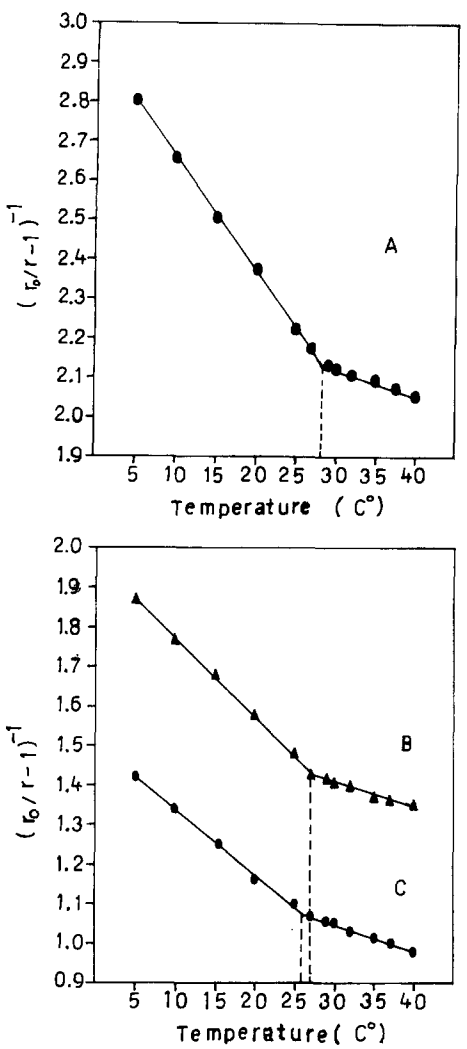

Fig. 1. Anisotropy parameter, $((r_0/r)-1)^{-1}$, versus temperature (in C°) plots of DPH-labeled liposomes prepared from BBMs of three gestational age groups. (A) 6–9 weeks (early), (B) 21–24 weeks (mid), (C) ≥ 35 weeks (term). From the breakpoints normals were drawn on temperature axis to find respective transition temperatures (T_m).

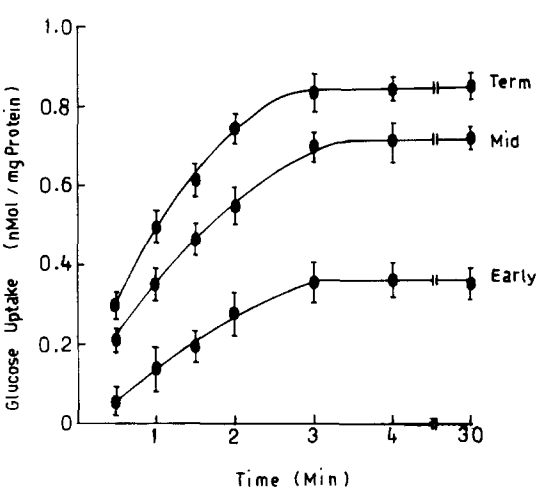

Fig. 3. Time-course for uptake of D-[^{14}C]glucose by BBMVs, prepared from early, mid and term placental BBMs. Values are means ± S.E. of four experiments.

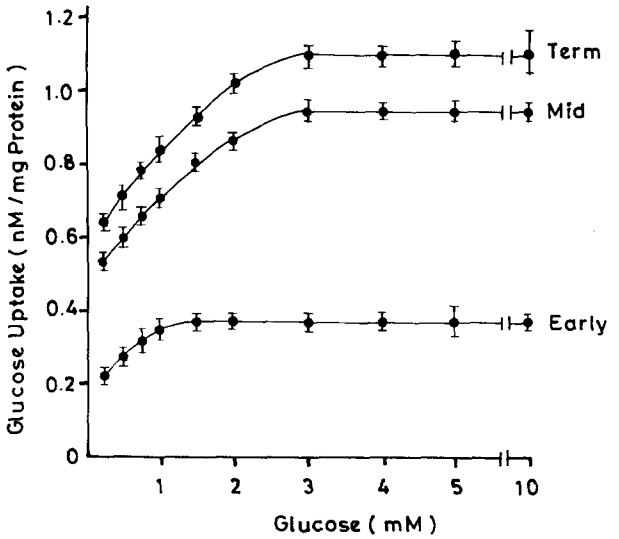

Fig. 4. The relationship between concentration of D-glucose and uptake over the first 5 min of incubation. BBMVs were added to the incubation medium containing the following final concentrations of ^{14}C-labeled D-glucose: 0.25, 0.5, 0.75, 1.0, 1.5, 2.0, 3.0, 4.0, 5.0 and 10.0 mM. Values are means ± S.E. of four experiments.

at very early gestational age (6–9 weeks) and the lowest at term.

SDS-PAGE pattern of BBM proteins is shown in Fig. 2, which reveals that there is no significant change among the BBM proteins at different gestational age groups. Even increasing the amount of protein loaded to each lane (E',M',T') no appreciable change could be observed.

Transport of glucose across the placental BBM was studied by monitoring the radiolabeled D-glucose uptake by BBMVs. Fig. 3 shows the time course for uptake of D-[^{14}C]glucose, while Fig. 4 represents the variation with different concentrations of substrate. The graphs for all three gestational age groups (early, mid and term) were of same nature with a steady increase in uptake upto a saturation point. Steady state glucose uptake increased 97.2% from early to mid gestational age, whereas the increase was only 16.9% from mid to term (Fig. 3). The 't' statistics between early and mid, and mid and term gestational age groups are denoted by t_1 and t_2, respectively. It has been found that $t_i > t_{0.05,18}$ $(i = 1,2)$, where $t_{0.05,18}$ denotes the upper 5% point of the distribution of 't' with 18 degrees of freedom [16]. So it can be concluded that glucose uptake increases significantly with advancement of pregnancy.

4. Discussion

Increase in total lipid and protein content of placental BBM (Table 1) with advancement of gestational age suggests incorporation of these two main membrane components in BBM formation. Lipids are able to interact with membrane proteins and thus regulate their function and/or activity. Alterations of the lipid composition of a membrane may result in changes of physicochemical properties and in modulation of enzyme activity in the membrane environment [11]. Cholesterol has an ordering effect on membranes which influences membrane associated properties such as membrane permeability, activities of membrane bound proteins and receptors, as well as membrane stability [17,18]. Cholesterol's 3(β)-OH, Δ^5 double bond, central planar ring and branched aliphatic chain at C-17 were selected by evolution to provide proper alignment with the phospholipid acyl chains and thus to modulate the fluidity parameters of lipid layers [19–21]. Considerable data indicate that the decrease in Ch/Pl ratio increases membrane fluidity [11,12,15]. Sterol induced fluidity changes begin to manifest themselves at Ch/Pl ratio above 1/10 [22]. The results of Table 1 suggest that with the progress of gestational age the fluidity of BBM increases as Ch/Pl ratio gradually decreases along the same period of time.

Much of the research on the role of lipid composition in biological systems, i.e., with intact cells or cell-derived membranes, has been carried out and interpreted against the background of responses of artificial membranes (liposomes). Fluidity studies with liposomes as membrane models have established the different determinants of lipid micro-viscosity, which is inversely proportional to fluidity, among these the ratio of Ch/Pl is an important factor [23]. Fluorescence polarization technique with DPH-labeled liposomes has been used in the present fluidity study because this technique has a series of advantages over ESR and NMR [23].

In warm blooded animals the bulk membrane Pl remains in the liquid-crystalline phase. Above the transition temperature (T_m), Ch lowers membrane fluidity by restricting the motion of fatty acyl chains. The function commonly ascribed to membrane-resident Ch is to order or decrease acyl chain mobility below the Pl T_m. At physiological temperatures and balanced Pl compositions, the ordering effect on acyl chain mobility probably predominates, at least as far as the bulk phase is concerned. In those membranes Ch may, however, have fluidising effects even at 37° C [22]. Thus we can explain the results of Table 2 where we find that with the progress of gestational age, r for both DPH-labeled liposomes and native BBMVs increases. This is an obvious consequence of decreasing Ch/Pl ratio with the advancement of pregnancy (Table 1).

In the present study, SDS-PAGE results (Fig. 2) indicate no contribution of the membrane proteins in the fluidity change of BBM as there is no detectable change in the gel pattern in the three gestational age groups [11].

The degree of fluidity is dependent on temperature besides the composition of the membrane [24]. This is evident from the results of Table 2, where we find a decrease in r for all the gestational ages with increase in temperature. Below the transition temperature membrane

lipids remain in a gel-crystalline state with the phospholipids restricted in their mobility and their hydrocarbon tails in continuous staggered conformations. As the temperature is increased, there is a phase transition into a liquid-crystalline state when the membrane lipids exist in a more mobile sol-phase with many of its hydrocarbon tails in skewed conformations. This transition results in an increased fluidity.

There are different levels of application of fluorescence polarization in fluidity determination. Shinitzky suggested that the anisotropy parameter $((r_o/r) - 1)^{-1}$ is better to serve as comparative scale for fluidity [23]. Thus, it was plotted against temperature to find out T_m (Fig. 1). Table 2 shows that with the advancement of gestational age T_m decreases. T_m has been found to be 26° C at term which is in conformity with the findings of Illsley et al. [25]. The T_m is not precise for all biological membranes; change in T_m for developing placental BBM may be due to its changing chemical composition (Table 1). Cullis and Hope suggested that T_m is profoundly influenced by Ch [26]. It is somewhat unusual to observe T_m at a relatively high mole ratio (> 0.3) of Ch at early gestational age as shown in Fig. 1A. However, in our study liposomes were prepared from placental BBM lipids comprising of Ch and different types of Pls instead of a single pure Pl. This may be a probable reason for finding T_m at early gestational age. It is also known that higher the proportion of Pl lower is the T_m. Since with increase in gestational age Ch/Pl ratio decreases, T_m also decreases. This change in T_m is further supported by the increased fluidity of BBM observed in our study [14].

In the present study the transBBM glucose transport with increasing gestational age was investigated with a view to find out any possible correlation between fluidity change and glucose transport. Fig. 4 shows that glucose transport at all the gestational age groups follows saturation kinetics. This result is in conformity with the findings of other investigators working with term placentas [15,27]. Glucose transport across BBM increases with advancement of pregnancy as is evident from Fig. 3. This may be due to the increasing demand of glucose during embryogenesis as very little gluconeogenesis occurs in the fetus [27]. Our study shows that with advancement of pregnancy fluidity of BBM increases which may be one of the reasons for increased transport of glucose across it. This is in agreement with the suggestion of Illsley et al. who showed that change in membrane fluidity influences glucose transport property of term placental BBM [25]. However, this proposition needs further kinetic analysis as well as a physical demonstration for confirmation. Alternatively, increase in membrane protein and lipid with gestational age (Table 1) may contribute to the formation of greater number of BBMV causing higher glucose uptake.

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References

[1] Labelle, E.F (1979) Biochim. Biophys. Acta 555, 259–269.
[2] Grassl, S.M. (1992) J. Biol. Chem. 267, 17760–17765.
[3] Ghosh, P.K., Das, T., Sa, G. and Mukherjea, M. (1992) Med. Sci. Res. 20, 705–706.
[4] Chaudhuri, D., Kushari, J. and Mukherjea, M. (1982) Eur. J. Obstet. Gynecol. Reprod. Biol. 13, 309–316.
[5] Balkovetz, D.I., Tirrupathi, C., Leibach, F.H., Mahesh, V. and Ganapathi, V. (1986) Am. J. Physiol. 251, C852–C860.
[6] Folch, J., Lees, M. and Sloane-Stanley, G.H. (1957) J. Biol. Chem. 226, 497–509.
[7] Sperry, W.M. and Brand, F.C. (1943) J. Biol. Chem. 150, 315–324.
[8] Higgins, J.A. (1984) Biochem. J. 174, 261–272.
[9] Fiske, C.H. and SubbaRow, Y. (1925) J. Biol. Chem. 66, 375–400.
[10] Lowry, O.H., Rosebrough, N.J., Farr, A.L. and Randall, R.J. (1951) J. Biol. Chem. 193, 265–275.
[11] Ohyashiki, T., Sakata, N. and Matsui, K. (1992) J. Biochem. 111, 419–423.
[12] North, P. and Fleischer, S. (1983) J. Biol. Chem. 258, 1242–1253.
[13] Shinitzky, M. and Barenholz, Y. (1974) J. Biol. Chem. 249, 2652–2657.
[14] Ohyashiki, T., Yamamoto, T. and Mohri, T. (1989) Biochim. Biophys. Acta 981, 235–242.
[15] Bissonnette, J.M., Black, J.A., Wickham, W.K. and Acott, K.M. (1981) J. Membr. Biol. 58, 75–80.
[16] Pearson, E.S. and Hartley, H.O. (1970) in Biometika Tables for Statisticians, Vol. 1, pp. 146, The Syndicate of the Cambridge University Press, Cambridge.
[17] Spector, A.A. and Yorek, M.A. (1985) J. Lipid Res. 26, 1015–1035.
[18] Muller, C.P. and Krueger, G.R.F. (1986) Anticancer Res. 17, 1181–1194.
[19] Demel, R.A. and De Kruijff, B. (1976) Biochim. Biophys. Acta 457, 109–132.
[20] Huang, C. (1977) Lipids 12, 348–356.
[21] Yeagle, P.L. (1991) Biochimie 73, 1303–1310.
[22] Bloch, K. (1985) in Biochemistry of Lipids and Membranes (Vance, D.E. and Vance, J.E., eds.), pp. 1–24, The Benjamin/Cummings Publishing Company, California.
[23] Shinitzky, M. and Barenholz, Y. (1978) Biochim. Biophys. Acta 515, 367–394.
[24] Devlin, T.M. (1986) in Text Book of Biochemistry (Devlin, T.M., ed.), pp. 177–210, John Wiley and Sons, New York.
[25] Illsley, N.P., Lin, H.Y. and Verkman, A.S. (1987) Biochemistry 26, 446–454.
[26] Cullis, P.R. and Hope, M.J. (1985) in Biochemistry of Lipids and Membranes (Vance, D.E. and Vance, J.E., eds.), pp. 25–72, The Benjamin/Cummings Publishing company, California.
[27] Johnson, L.W. and Smith, C.H. (1980) Am. J. Physiol. 238 (Cell Physiol. 7), C160–C168.