Global Journal of Medical Research
Volume 12 Issue 9 Version 1.0 Year 2012
Type: Double Blind Peer Reviewed International Research Journal
Publisher: Global Journals Inc. (USA)
Online ISSN: 2249-4618 Print ISSN:0975-5888

Effect of Membrane Cholesterol on Glucose Uptake in Diabetic Erythrocytes

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In order to check this hypothesis, we studied the effect of media cholesterol concentration on the erythrocyte membrane cholesterol levels as well as the effect of such an altered membrane cholesterol level, if any on glucose uptake in diabetic erythrocytes. Erythrocytes derived from type 2 diabetic subjects were incubated in cholesterol rich albumin medium for a period of 2 hours and amount of cholesterol included on the erythrocyte membrane was estimated in washed incubated erythrocytes along with glucose uptake, lactic acid production and glycolytic index were studied.

Keywords : *Type 2 diabetes, membrane cholesterol, Glucose uptake.*

GJMR-B Classification : *NLMC Code: WK 825, WK 840, WK 870*

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In order to check this hypothesis, we studied the effect of media cholesterol concentration on the erythrocyte membrane cholesterol levels as well as the effect of such an altered membrane cholesterol level, if any on glucose uptake in diabetic erythrocytes. Erythrocytes derived from type 2 diabetic subjects were incubated in cholesterol rich albumin medium for a period of 2 hours and amount of cholesterol included on the erythrocyte membrane was estimated in washed incubated erythrocytes along with glucose uptake, lactic acid production and glycolytic index were studied. The results suggests that there is a significant increase in cholesterol inclusion (N=3.78 ± 0.38, T2DM= 4.13±0.09, $p<0.001$), a significant decrease in glucose uptake (N=2.12±0.96,T2DM=0.79±0.28, $p<0.001$), lactic acid production(N=0.24 ± 0.10, T2DM= 0.16±0.07 $p<0.001$), percentage of glucose uptake(N=18.97 ± 7.20, T2DM= 7.07±2.80, $p<0.001$),and glycolytic index(N=11.04 ± 1.04, T2DM= 4.24±2.05, $p<0.001$) in erythrocytes of type 2 diabetic subjects. Suggesting a positive effect of media cholesterol on erythrocyte membrane cholesterol in diabetic erythrocytes.

Keywords : Type 2 diabetes, membrane cholesterol, Glucose uptake.

I. Introduction

The most common biochemical alterations observed in type 2 diabetes mellitus is decreased utilization of glucose, which may be due to subnormal insulin amount or suboptimal function of insulin. The most relevant findings are hyperglycemia and glucosuria with changes in lipid as well as protein metabolism.

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Cholesterol is essential for maintenance of the structural and functional integrity of all biological membranes, including erythrocytes membrane and it plays a key role in maintenance of the bilayer matrix in an intermediate fluid state. (1). The decreased utilization of glucose by tissue cells as well as by the erythrocytes seen in diabetes mellitus may be due to decreased transport of glucose into the cells which is purely a function of erythrocyte membrane. Though the glucose transport is facilitated by glucose transporter (GLUT) presents in membrane, whose action may be influenced by insulin, the role of membrane lipids specifically phospholipids and cholesterol cannot be ignored.

The relative amounts of phospholipids and cholesterol are responsible for the fluid properties of the erythrocyte membrane (11) and for the shape as well as basic structural integrity of erythrocyte. An alteration in membrane lipid composition may bring about certain changes in glucose transport. The increased membrane cholesterol content, increased saturated fatty acid content was observed in diabetic erythrocyte membrane (8). The diabetes induced hyperglycation of membrane proteins including related GLUT particles may induce changes in distribution of membrane lipid components as well as may induce certain changes in membrane transport activity (2) possibly including glucose transport.

Present study was undertaken to establish the effect of incubation media cholesterol concentration on erythrocyte membrane cholesterol content as well as to establish the effect of such included cholesterol, if any, on the glucose transport in type 2 diabetic erythrocytes.

II. Materials and Methods

Diabetic type 2 subjects (male and female) in the age group of 30-60 years attending Medical OPD of Basaveshwara Medical College Hospital and Research Center, Chitradurga, were randomly selected.

The normal subjects (male and female) were randomly picked among house surgeons and employees of the college as well as Hospital, who were in the age group of 30-60 years.

Blood samples (6-7ml) from the selected normal subjects and type 2 diabetic subjects were collected, in the fasting state, with heparin as an anticoagulant after obtaining informed consent. Plasma was separated by centrifugation at 3500 rpm, for 10

minutes. Erythrocytes were washed three times with an aliquot of 5 ml normal saline and then were mixed with equal volume of normal saline so as to give 50% saturated erythrocyte suspension. This erythrocyte suspension was used in the present studies.

III. Cholesterol Inclusion Studies

Cholesterol- enriched- albumin solution was used as a cholesterol donor in the present study. (1 gram of fine powered cholesterol in 100 ml 1% albumin solution). Cholesterol content of this media was determined by triplicate estimation of cholesterol (7).

1ml of 50% saturated erythrocytes both normal/ diabetic were separately incubated with 0.6 ml of cholesterol rich albumin medium at 37°C in a temperature controlled water bath for 2 hours. After stipulated incubation period, the erythrocytes were washed with 3 times with 3ml aliquot of normal saline. One part of washed erythrocytes was mixed with 4 ml distilled water, the mixture stirred vigorously with a clean glass rod to lyse the erythrocytes. This was centrifuged at 3500 rpm for 5 minutes. Supernatant was discarded. The sedimented membranes were washed 3 times with 3 ml aliquot of normal saline. The resultant membranes were mixed with 9 parts of chloroform: methanol mixture (1:1 v/v) and homogenized for 8 minutes in a Potter-Elvejham tissue homogenizer. The extracts were used for estimation of membrane cholesterol (7).

The rest of the erythrocytes incubated with cholesterol-rich-albumin medium, were employed for glucose uptake studies.

IV. Studies on Glucose Uptake by Erythrocytes and Lactic Acid Production

To 0.5 ml of cholesterol-rich-albumin medium was incubated erythrocyte of both normal and diabetic subjects were separately mixed with 0.5 ml of normal saline, 1 ml of 0.1% freshly prepared aqueous glucose solution was added to both. An aliquot of 0.5 ml mixture was immediately pipette out into a tube marked N_o and D_o containing 4 ml of 10% TCA, the contents were mixed and centrifuged at 3500 rpm for 5 minutes and the supernatants were employed for estimation N_0 and D_0 minute glucose content (10) and lactic acid contents (3). The rest of the erythrocyte mixture was incubated in temperature controlled water bath at 37°C for 1 hour. At the end of the incubation time another aliquot of 0.5 ml mixture was pipette out into a tube marked N_{60} and D_{60} and proceeded as above. The supernatants were used for 60 minutes glucose and lactic acid estimation in normal and diabetic erythrocytes.

The data obtained was statistically evaluated using students't 'test.

V. Results

In the present study, a total number of 192 subjects were employed, which include 52 normal subjects and 140 diabetic subjects. The normal subjects were consisted of 44 male subjects and 08 female subjects. Further diabetic group consisted of 85 male diabetic subjects and 55 female diabetic subjects. The results of the present study are narrated in table 1and 2. Table 1 gives, glucose uptake, percentage of glucose uptake, lactic acid production, as well as glycolytic index in erythrocytes of normal subjects and in erythrocytes of diabetic subjects. As seen from the table there is a significant decrease observed in glucose uptake ($p<0.001$), percentage of glucose uptake ($p<0.001$), lactic acid production ($p<0.001$), as well as glycolytic index ($p<0.001$) in erythrocytes of diabetic subjects as compared to normal subjects, indicating there is a decrease in glucose uptake and utilization in diabetic erythrocytes.

Table 2 depicts erythrocyte membrane cholesterol prior to the incubation and post incubation with cholesterol rich albumin medium, as well as glucose uptake by these erythrocytes. It is evident from the table that there is a significant elevation in cholesterol inclusion on both normal as well as diabetic erythrocytes which are exposed to cholesterol rich medium, as compared to non- exposed erythrocytes ($p<0.001$). Further it is evident from the table that, the glucose uptake is decreased ($p<0.001$) in cholesterol rich albumin medium exposed erythrocytes (normal/diabetic) as compared to non-exposed counter parts. This decrease in glucose uptake may probably due to extra cholesterol included onto the membrane.

VI. Discussion

The membrane surrounding the erythrocyte serving as a barrier, the membrane contains pumps and channels for the movements of sodium, potassium and calcium and it facilitates the transport of glucose and other small molecules. It is also responsible for the basic structural integrity of the erythrocytes. A decreased utilization of glucose by the tissue cells in type 2 diabetes mellitus is attributed to either lack of insulin or due to non-availability of functioning insulin (13).

Increased cholesterol and phospholipid contents in erythrocyte have been correlated with decrease in erythrocyte membrane fluidity in diabetes mellitus and these parameters identified as contributing factors for decrease in membrane fluidity (5). This erythrocytes membrane lipid alteration may be due to diabetes induced dyslipidemia. An increase in cholesterol may induce rigidity into the membrane, whereas increase phospholipid induces more flexibility. In addition probably the glycation of membrane proteins including related GLUT particles may induce changes in distribution of membrane lipid components as well as

may induce certain changes in membrane transport activity (4).

In the present study, a significant raise in the inclusion of cholesterol ($p<0.001$), onto the erythrocyte membrane as been observed in erythrocytes which are incubated with cholesterol rich albumin medium compared to non-exposed erythrocytes. This is in agreement with the reports of Christopher (6) and Steven (12). When the erythrocyte which are incubated with cholesterol rich albumin medium were used for glucose uptake studies, it was found that there is a significant decrease in glucose uptake ($p<0.001$), percentage of glucose uptake ($p<0.001$), lactic acid production ($p<0.001$), as well as glycolytic index($p<0.001$), in erythrocyte of type 2 diabetic subjects. This suggests that an increase in cholesterol content of erythrocyte membrane may result in decreased glucose uptake, which may partly due to an alteration in membrane lipid composition, leading to altered membrane proteins orientation, possibly GLUT particles, which may cause a decrease in glucose uptake in these erythrocytes.

In conclusion it can stated that, when erythrocytes (normal/diabetic) exposed to cholesterol rich albumin medium, an extra cholesterol migrate onto the membrane (9), resulting in increase of membrane cholesterol level. Further such an increase in membrane cholesterol level decrease significantly glucose uptake by these erythrocytes.

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Table 1 : Showing glucose uptake, percentage of glucose uptake, lactic acid production and glycolytic Index in normal erythrocytes as well as in diabetic erythrocytes.

Groups → / Parameter ↓	Erythrocyte of normal subjects (n = 36)	Erythrocyte of diabetic subjects (n = 90)
Glucose uptake by erythrocyte mg/cc	2.12 ± 0.96	0.79*** ± 0.28
Percentage of glucose Uptake	18.97 ± 7.20	7.07*** ± 2.80
Lactic acid production mg/cc	0.24 ± 0.10	0.16*** ± 0.07
Glycolytic index	11.04 ± 1.04	4.24*** ± 2.05

Note : 1.The number in parenthesis shows the number of samples.
2. Values are expressed as their Mean ± SD.
3. p- value * $p<0.05$, ** $p<0.01$, *** $p<0.001$.
4. Glycolytic index = $\frac{\text{Glucose uptake mg/cc erythrocytes}}{\text{Lactic acid production mg/cc erythrocytes}}$

Table 2 : Showing erythrocyte membrane cholesterol and glucose uptake in normal erythrocytes as well as in diabetic erythrocytes both prior and post incubation in cholesterol rich albumin media.

Groups → / Parameter ↓	Erythrocyte of normal subjects (n = 16)	Erythrocyte of diabetic subjects (n = 50)
Erythrocyte membrane cholesterol prior to the incubation mg/cc	1.25 ± 0.31	1.52*** ± 0.13
Erythrocyte membrane cholesterol after incubation mg/cc	3.78 ± 0.38	4.13*** ± 0.09
Glucose uptake by erythrocytes prior to the incubation mg/cc	2.12 ± 0.38	0.79*** ± 0.26
Glucose uptake by erythrocytes after incubation mg/cc	1.66 ± 0.47	0.38*** ± 0.15

Note: 1.The number in parenthesis shows the number of samples.
2. Values are expressed as their Mean ± SD.
3. p- value * $p<0.05$, ** $p<0.01$, *** $p<0.001$.