High Protein Carbohydrate Free Diet and Wound Healing in Experimental Diabetes

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Introduction

Delayed wound healing is a recognised phenomenon in diabetes mellitus[1,2]. The poor healing in the diabetics can be explained in terms of delayed as well as diminished early inflammatory response to injury[3]. The impaired healing can be restored back to normal by controlling diabetic state[4]. Recently, a high-protein cabohydrate (HP CHO) free diet is reported to improve drug induced diabetes mellitus in rats with in a short period[5]. Dietary protein is also reported to improve wound healing in the malnourshed[6]. However, no attempt has been made uptill now to observe the role of HP CHO-free diet on wound healing in experimental diabetes in rats. Keeping these facts in mind, the present study was undertaken to investigate wether a HP CHO- free diet can improve wound healing in experimental diabetes.

Materials and Methods

Seventy seven long Evans rats of both sex were taken. The age of the rats ranged between 2 to 6 months. They were allowed an initial period of 3 to 4 weeks for adaptation. During this period they were provided with a normal laboratory diet (12% Protein, 7% Fat and 58% Carbohydrate). At the onset of the experiment their body weight was recorded and blood glucose levels were estimated. They were randomly divided into two groups. In the majority (n=52) of the rats diabetes was induced by injecting streptozotocin (STZ) (vehicle-normal saline) at a dose of 50mg per kg body weight. The other group (n=25) (Group C) was injected equal volume of isotonic normal saline compared to those treated with STZ. Forty eight hours after injection, body weights were recorded and blood glucose levels were estimated. On the next day of confirmation of diabetes a 4 cm long incised skin deep wound was inflicted on the back of each rats and the wound was closed by intermittent silk suture at an intervals of one centimeter to attain healing by first intention. A tincture benzoine seal was applied to protect the wound from contamination and also from the biting of their fellow rats . Each rat also received single dose of injection penicillin. The diabetic rats were then randomly divided into two groups. Rats of one group (n=26) (Group A) was provided with a HP CHO free diet (70% Protein & 8% Fat. [5,7,11]) (Table 5). The other diabetic group (n=26) (Group D) along with the normal control group (Group C) were provided with normal

Bangladesh Journal of Nutrition Vol. 3, No. 1 & 2 1-11 , Dec.1989- June 1990 Printed in Bangladesh.
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laboratory diet. Body weight of therats were recorded at an intervals of 3 days. A few drops of urine was collected from each rat and their urinary glucose excretion were also measured at an interval of three days by using glucotest test strip (Boehringer Manheim). Blood glucose levels were estimated (Haemogluco-test, Boehringer Manheim) on the 5th, 14th, 21st, 28th, 35th, and 42nd post-oparative day. The amount of diet consumed by different groups of rats were also evaluated. The rats were sacrificed on the 5th, 14th, 21st and 42nd post-oparative day in different batches (I, II, III & IV). Wound healing was assessed through mesurement of the tensile strength (figure 3) of the wound [8]. Tensile strength is the force necessary to break a wound taking the sectional area of the wound into account. Histological examination of the wound was also done. The healing of the wounds of the three groups were compared.

Results

Diet Consumption

Daily food consumption of the rats of group D was highest (Table 1) and lowest by the rats of group A.

After STZ injection there was an initial weight loss (Figure 1) observed in the rats of group A and D. There after, the rats of Group A had a steady weight gain but the the rats of Group D failed to show any substantial weight gain.

Table 2 shows the mean blood glucose levels of the different Groups of rats at different intervals of time.

On the second day after STZ injection, before transfer of Group A to the high-protein carbohydrate free diet, excretion of glucose in urine was same (Figure 2) in all the diabetic rats of Group A and D. After that, excretion of glucose in Group D rose to much higher levels than in the animals of Group A. Urinary glucose excretion reduced gradually and eventually became nil in Group A.

Macroscopic appearance of the wounds

The gross appearance of the wounds of the rats of Group A and C showed good apposition but those of rats of Group D showed signs of inflammation although showed good apposition. On the 14th, 21st, and 42nd day the wounds of the rats of all three groups were normal. Table 3 shows mean tensile strength of the wounds of the rats of different groups.

Histologic examination of the wounds

Representative sections of wounds of each of the rats of each batch and each groups were examined. Both H&E and Masson trichrome (MT) stained sections were examined. The status of healing was categorized into three groups i.e. Good healing, intermediate healing and Poor healing for each batch of rats.

1st batch: 5th day wound

Good healing : There was complete epithelialization of the epidermal gap.

All four layers of epidermis were distinctly recognised. The dermal gap was filled with abundant granulation tissue (Figure 4). H&E stained sections presented numerous fibroblasts, histiocytes and lymphocytes. Less numerous were polymorphonuclear leucocytes and eosinophils. A large number of endothelial cells were seen forming vascular channels. The interstitial oedema was minimum. MT section showed scattered collagen fibrils in the granulation tissue.

Poor healing : Although there was complete epithelialization of the epidermal incisional space, but the amount of granulation tissue was very small (Figure 5). Scanty fibroblasts were seen arranged irregularly. Histiocytes and lymphocytes were minimum. Polymorphs and eosinophils were also minimum in number. Considerable interstitial oedema was seen. Neovascularisation was remarkably less. MT stained section failed to reveal collagen fibrils.

Intermediate healing: Any wound having charecteristics in between *Good healing & Poor healing* was considered as *intermediate healing* (for all batches).

2nd batch: 14th day wound

Good healing : The epithelium was near normal in thickness. The dermal incisional gap was filled with well contracted scar. In contradistinction to the 5th day wound, the cellular reaction was less numerous. Young fibroblasts and vascular channels were more numerous than uninvolved dermis. MT stained sections revealed collagen fibrils arranged in loose fascicles.

Poor healing : The epithelium was near normal in thickness. The dermal gap was filled with profound granulation tissue. Numerous fibroblasts, histiocytes and lymphoctes were seen in the granulation tissue. Polymorphonuclear leucocytes and eosinophils were also seen in considerable numbers. There was also interstitial oedema. Abundant vascular channels were seen lined by endothelial cells. MT stained section showed scanty collagen fibrils in the granulation tissue.

3rd batch : 21st day wound

Good healing : The wound was slightly more cellular than the nearby uninvolved dermis. Within the wound the collagen fibrils were arranged in loose interlacing fascicles staining pink to red. MT stained sections showed blue collagen fibrils arranged in loose fascicles in the wound.

Poor healing : Abundant granulation tissue was present. Numerous fibroblasts, histiocytes and lymphocytes were seen in the granulation tissue. A few polymorphonuclear leukocytes were seen. Abundant vascular channels were also seen. MT stained sections revealed scanty collagen fibrils present in the granulation tissue.

4th batch: 42nd day wound

Good healing : The wound was relatively acellular and collagen deposition was extensive. The fibrils were numerous, deep, large and compact in H & E stained sections though their fibrillar character was more easily recognisable than in the nearby intact collagen . Vascularity of the wound was about the same as in the uninvolved dermis.

Table 1. *Amount of diet in gram consumed per 100 gram of rats at different intervals of time.*

Day	Normal Control C	n= 25	Diabetic HP CHO A	n= 26	n=52 Control D	n= 26
05th.	10.44	n=6	7.18	n=6	12	n=6
14th	10	n=6	7.54	n=6	15.73	n=6
21st	10.50	n=6	7.96	n=7	17.77	n=7
42nd	10	n=7	9.57	n=7	18.91	n=7

Table 2. *Mean blood gloucose level (in mg / dl) of different groups of rats at different time interval.*

Groups	Before STZ	After STZ	5th day	14th day	21st day	28th day	35th day	42nd day
Protien A	82.46	398.37	255.73	131.85	131.85	128.58	128	127.14
Normal * C	82.46	82.60	82	81	81.92	82.85	83.28	81.85
diabetic D	82.46	398.37	404.19	392.9	383.57	398.85	409	418.85

* Rats of group C were not injected with STZ (streptozotocin).

Table 3. *Mean tensile strength in different groups of rats*

Day	Normal Control C	Diabetic HP CHO: A	Diabetic Control D	F value	P value
5th	21.67	19.51	15.64	2.3102	N.S.
14th	62.74	54.06	40.65	12.390	<.01
21st	145.95	118.14	65.57	25.925	<.01
42nd	532.76	449.77	249.80	271.32	<.01

*N.S. = Not Significant * HP CHO = High Pritien Carbohydrate free diet.

Table 4. *Status of healing in different groups of rats.*

Batch	Day	Group	Total	Good	Inter	poor
I	5th	A	6	2	4	0
		C	6	6	0	0
		D	6	0	1	5
II	14th	A	6	4	2	0
		C	6	5	1	0
		D	6	1	0	5
III	21st	A	7	4	2	1
		C	6	6	0	0
		D	7	1	1	5
IV	42nd	A	7	4	3	0
		C	7	6	1	0
		D	7	0	1	6

Table 5. *Composition of High Protein Carbohydrate free diet (excluding water)*

Casein (sigma)	: 70gm
Corn Oil	: 08gm
Fiber (cellulose)	: 15gm
Vitamins and Salt (BPI)	: 07gm
Percentage of calories from different sources	
Protien	: 80%
Fat	: 20%
Carbohydrate	: Nil

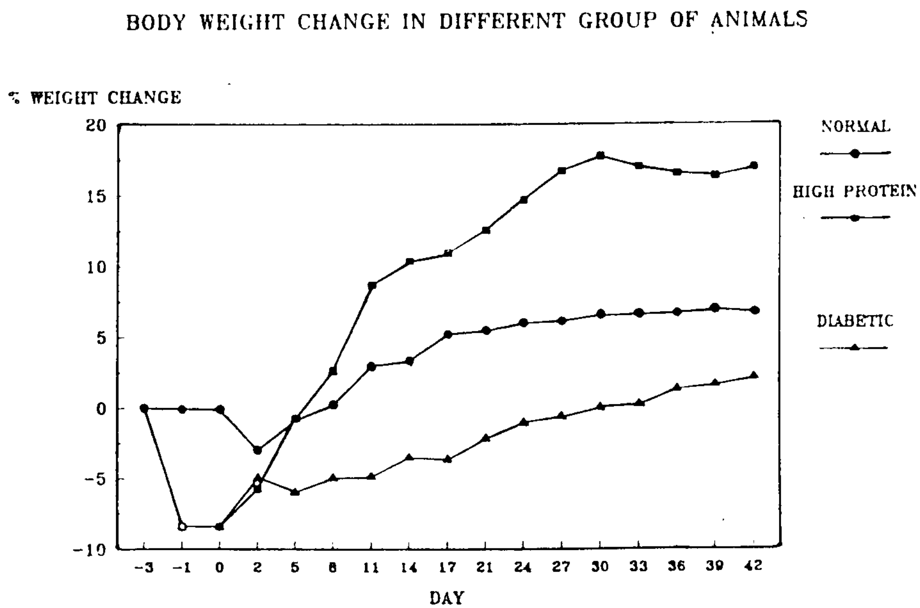


Figure 1 : Body weight changes in gm/ 100gm of rats in different groups of rats.

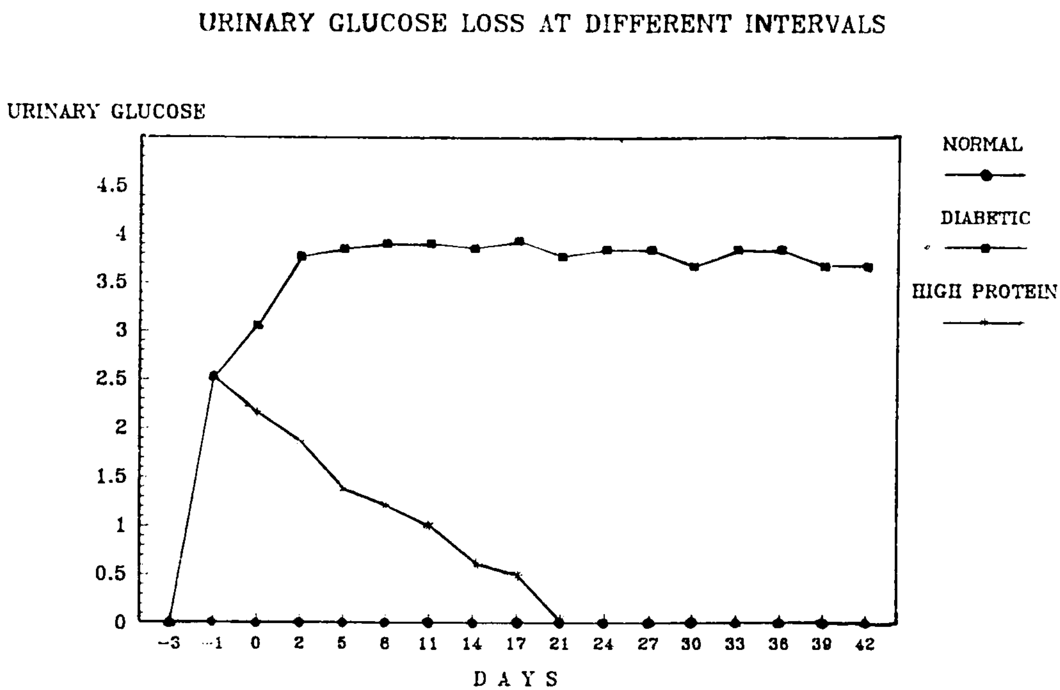
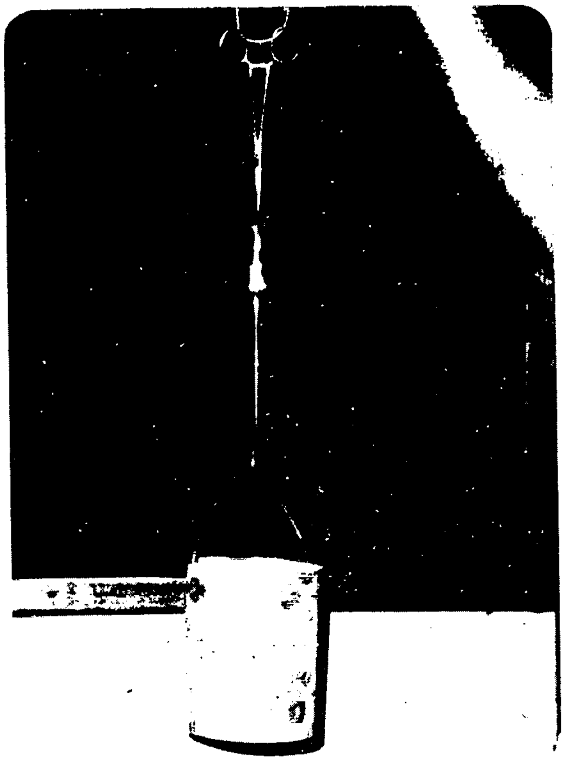


Figure 2 : Urinary glucose loss in gm/ dl in different groups of rats.



Figire 3 : Measurement of tensile strength of wound of skin of rats.

Figure 4 : Histologic section of the 5th day wound. Example of good healing.

Figure 5 : Histologic section of the 5th day wound. Example of poor healing.

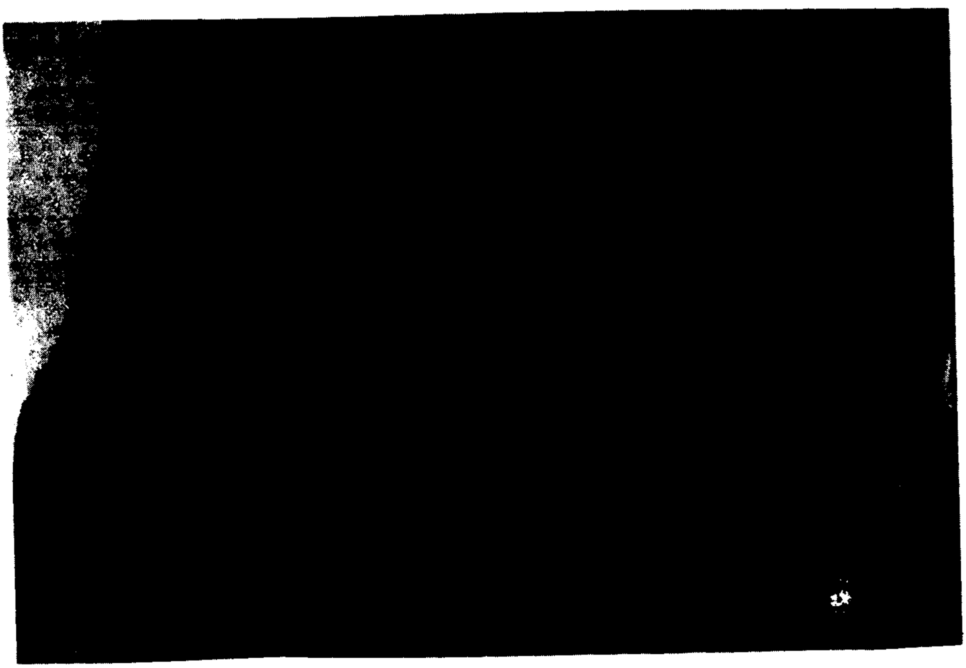


Figure 6 : Histologic section of the 42nd day wound. Example of poor healing.

Poor healing : Profound granulation tissue containing numerous fibroblasts was the hallmark (Figure 6). A few histiocyties and lymphocytes were present. Vascular channels were abundant. Iinterstitial oedema was minimum. Moderate number of collagen fibrils were seen in the granulation tissue. MT stained sections showed collagen fibrils arranged in loose fascicles.

Using this criteria each of the wounds of different batches of different groups of rats were examined by an observer without prior knowledge of the origin and batches of the animals and tabulated in table 4.

It is apparent from table 4 that rats of *Group C* had good healing and rats of *Group D* poor healing. Rats of *Group A* had healing nearer to *Group C* .

Discussion

The data presented here indicates that the short term use of a HP CHO free diet can greatly improve wound healing in rats previously treated with STZ in addition to amelioration of diabetic state. Previously Eizirik and associates[5] reported amelloration of diabetes within a short period by using the same diet. The mechanism by which a HP CHO free diet ameliorates diabetic state is still not clear. But the probable mechanisms include firstly an immediate effect of absence of CHO in diet [5,9,10,11,12] which helps to put the beta cells in a state of lower functional activity[11] and thereby helps in improving islet cell function[13] . Since the beta cells get a better chance of recovery due to lower demand on its functional capacity. Secondly, the elevated levels of plasma BCAAs[14,15,16] may help the beta cells in repairing DNA damage caused by STZ and thereby restoration of function and finally systemic anticatabolic effect of BCAAs. It is apparent from table 3 and table 4 that healing of wound of rats on HP CHO free diet show significant improvement than that of the diabetic rats on normal diet. There are a lot of report that controlling of diabetic state by insulin., wound healing can be restored back to normal. Insulin initiates the early inflammatory response following injury[17] and by controlling the hyperglycemic environment it helps in collagen cross linking[18]. Dietary protein is utilized by the body for increased plasma protein and acute phase protein synthesis following injury. Powanda and Moyer[19] emphasized the versatile role of acute phase protien and plasma protien in wound healing. During extravasation in the wound albumin acts as a transport agent of amino acids, free fatty acids, carbohydrates, hormones and zinc. These nutrients are utilized by fibroblasts for synthesis of collagen and membrane lipid. Acute phase protiens help in various steps of repair process i.e. alignment of structural protien, collagen synthesis and crosslinking by carrying amino acids and copper, organization of celluar elements associated with removal of damaged tissue and modulation of hormonal and immunological response. Among the amino acids, cystine[6] and methionine[20] are particularly known to stimulate collagen synthesis and ground subtance formation.

Arginine[21] is known to improve healing by induction of inflammatory response, neovascularisation, celluar proliferation and collagen synthesis.

In the present study tensile strenght of wounds (table 4) of rats of *Group A* was significantly higher than that of *Group D* from the 14th day onwards but not on 5th day. It is the time when collagen synthesis has already been established and tensile strength is dependent on collagen synthesis. So it is more likely that HP diet helped in collagen synthesis in these rats.

Summary

The problem of delayed wound healing in diabetes can be overcome by controlling diabetes. Recently it has been reported that high protein carbohydrate free diet ameliorate the diabetic state without insulin in experimentally induced diabetes mellitus in rats. The present study aims at determining the effect of such a diet on wound healing in diabetic animals. Incised skin deep wound was inflicted on both normal and streptozotocin induced diabetes in rats and the wounds were sutured to attain healing by first intention. Sequential stages of healing in high protein cabohydrate free diet fed rats was compared with those in normal healthy and untreated diabetic rats with similar wounds. The diabetic state was assessed through blood glucose, urinary glucose and body weight measurment. Healing was assessed by tensile strenght mesurment and histology. From the 14th day of the experiment, the tensile strength of high protein carbohydrate free diet fed animals significantly differed from that of the diabetic animals. It therefore, appears that high protein carbohydrate free diet helps in diabetic wound healing.

Acknowledgement

This study was carried in the Deperment of Pathology, I.P.G.M.R, Dhaka in the year 1988-89. The authors gratefully acknowkedge the generous support of Fisons Bangladesh Limited. Please contact Dr. Md. Golam Mostafa for reprint.

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