ELSEVIER

Journal of Nutritional Biochemistry 13 (2002) 522–527

THE JOURNAL OF Nutritional Biochemistry

Modulatory effect of butyric acid—a product of dietary fiber fermentation in experimentally induced diabetic rats

Chethan M. Kumar, Kollegal S. Rachappaji, Chilkunda D. Nandini, Kari Sambaiah, Paramahans V. Salimath*

Department of Biochemistry and Nutrition, Central Food Technological Research Institute, Mysore 570 013, India

Received 5 October 2001; received in revised form 16 January 2002; accepted 11 February 2002

Abstract

The effect of feeding of butyric acid on alleviation of diabetic status was studied. Diabetes was induced in rats using streptozotocin. Rats were fed with basal diet containing wheat bran (5%) as a source of insoluble dietary fiber and guar gum (2.5%) as a source of soluble dietary fiber. The experimental group received butyric acid at 250, 500 and 750 mg/kg body weight/day. The diabetic animals lost weight in spite of high diet consumption. The levels of water intake, urine output, urine sugar, fasting blood sugar increased during diabetic condition compared to control and these were reduced by nearly 20% in the fiber-fed diabetic group. Further supplementation of butyric acid at 500 mg/kg body weight/day ameliorated the diabetic status by nearly 40%. Urine sugar level during the diabetic state was reduced from 7.2 g/day to 3.6 g/day and fasting blood glucose from 270 mg/dl to 180 mg/dl. Butyric acid feeding at 500 mg/kg body weight/day was most effective in controlling the diabetic status.

Keywords: Diabetes; Dietary fiber; Butyric acid; Wheat bran; Guar gum

1. Introduction

Diabetes mellitus is a metabolic disorder characterized by chronic hyperglycaemia associated with absolute or relative deficiencies in insulin secretion or function. Nephropathy, Neuropathy and Retinopathy are among the associated problems [1]. During diabetic nephropathy, the glomerular basement membrane becomes thicker [2], making glomeruli more porous to passage of macromolecules. Analysis of glomerular basement membrane during diabetes has indicated changes in its constituents, with an increase in the major component–type IV collagen and reduction in laminin and heparan sulfate proteoglycan [3–5].

Diet plays a major role in the management of diabetic complications. Dietary fiber, broadly defined as unabsorbable carbohydrates in foods, has many effects in the gastrointestinal tract, including altering fluid dynamics, slowing macromolecule digestion, and absorption of nutrients, etc. The beneficial role of dietary fiber against a variety of diseases including diabetes is well established [6,7]. Slow absorption of glucose by dietary fiber present in the intestine is well documented and understood [8,9]. Further these dietary fibers are fermented by the microflora present in the colon to short chain fatty acids [10]. Levels of short chain fatty acids including butyric acid formed from different dietary fibers have been measured in blood [11,12]. The role played by butyric acid—a four-carbon fatty acid in particular, on various physiological functions has received great attention [13,14]. Recent research has revealed a number of functions of butyric acid. Its ability to modify nuclear architecture and induce cell death by apoptosis in colon cancer is one of them. Butyric acid is shown to have potential in decreasing the incidence of bowel cancer, which is associated with a decreased fiber intake [15,16].

Butyric acid in particular is known to modulate activities of many key regulatory enzymes [17], including enzymes involved in glycoconjugate metabolism [18,19]. Derivatives of butyric acid having different functional properties are also gaining lot of interest, and some of them are undergoing clinical trials for tumor therapy. One of the derivatives of butyric acid—JTT-608 (4-trans-4-methyl cyclohexyl-4-O-oxobutyric acid) is shown to selectively reduce glucose levels in diabetic rats [20]. In *in vitro* studies using a cell culture system, sodium butyrate was shown to induce insu-

* Corresponding author. Tel.: +0091-821-514876; fax: +0091-821-517233.

E-mail address: paramahans1954@yahoo.com (P.V. Salimath).

0955-2863/02/$ – see front matter
PII: S0955-2863(02)00180-8

Table 1
Composition of basal AIN-76 diet

Composition	SFC/SFD	FFC/FFD
	g/kg diet	
Casein	200	200
AIN-76 vitamin mixture	10	10
AIN-76 mineral mixture	35	35
Choline chloride	2	2
Fat	100	100
Corn starch	653	578
Wheat bran	—	50
Guar gum	—	25

lin gene expression [21]. Recent studies have shown that butyrate modulates genes at the transcriptional level by histone acetylation [22].

Though butyric acid plays an important role in modulation of many disease conditions, its beneficial effect on diabetes is not well known. In this investigation an attempt is made to study the beneficial effect of butyric acid over and above moderate levels of dietary fiber on diabetic status in experimentally induced diabetic rats.

2. Materials and methods

2.1. Animals and diet

Male Wistar rats weighing between 110–120 g were placed into two main groups. The first group received a starch-based diet without fiber and second group received a diet supplemented with fibers. Each group was subdivided into control and diabetic groups. The control group had 6 animals while the diabetic group had 14 animals. The experimental group received butyric acid at 250, 500 and 750 mg/kg body weight/day in drinking water. Composition of basal AIN–76 diet is in Table 1 [23].

2.2. Induction of diabetes

Streptozotocin (Sigma, St. Louis, USA) dissolved in freshly prepared citrate buffer (0.1 M, pH 4.5) was injected intraperitoneally at a concentration of 55 mg/kg body weight [24]. Control animals were injected with citrate buffer only. Following streptozotocin injection, the rats were given 5% glucose solution overnight.

2.3. Butyric acid feeding

Known amount of butyric acid (SRL, Analytical grade, Mumbai, India) obtained commercially was given in drinking water via feeding bottles. From the quantities of water containing butyric acid consumed by the rats, the amount of butyric acid and water consumption were calculated. Butyric acid feeding was started after a week of streptozotocin injection.

2.4. Collection of blood and urine samples

Blood was drawn from the retro-orbital plexus into tubes containing heparin (20 U/ml blood) for measuring fasting blood glucose. The urine output was measured by collecting urine after keeping the rats in metabolic cages. The urine was collected under a layer of toluene.

2.5. Measurement of fasting blood glucose and urine sugar

Glucose in blood was measured by the glucose oxidase method [25]. The reducing sugar in urine was measured by the dinitrosalicylic acid method [26].

2.6. Statistical analysis

Comparisons between control, diabetic and treated groups were performed with a Student's *t*-test [27]. p-Values of less than 0.05 were considered to be significant.

3. Results

Male Wistar rats weighing between 110–120 g were maintained on diets containing starch in the control group, wheat bran (5%) and guar gum (2.5%) in the fiber-fed groups. Diabetes was induced using streptozotocin. Butyric acid was given to rats in the drinking water. Various concentrations of butyric acid (1, 5, 10, 50, 100, 250, 500, 750 and 1000 mg/kg body weight/day) were given to diabetic rats. Of these concentrations, butyric acid feeding at 250, 500 and 750 mg/kg body weight/day showed promising results and hence were taken for further studies. The rats were monitored for dietary intake, water intake, butyric acid intake, urine output and urine sugar. The rats were sacrificed under ether anesthesia when mortality was about to set in, which was 45 days after the streptozotocin injection. Mortality started initially in the starch-fed diabetic group. A night before sacrificing the rats, they were fasted and blood was collected to measure fasting blood sugar.

3.1. Effect of butyric acid on dietary intake and gain in body weight in control and diabetic rats

The dietary intake was greater in diabetic rats compared to control rats (Table 2). The starch-fed control animals consumed about 13.0 $\pm$ 0.49 g/day whereas fiber-fed control animals consumed 13.2 $\pm$ 1.35 g/day which was comparable. The greater amount of dietary consumption in the diabetic group was due to the hyperphagic condition developed during diabetes. Greater amounts of diet were consumed by fiber-fed diabetic rats compared to starch fed

Table 2
Effect of butyric acid on dietary intake and gain in body weight in control and diabetic rats

Groups	Diet intake (g/day)	Initial body weight (g)	Final body weight (g)	Gain in weight (g)
SFC	13.0 ± 0.49	116 ± 4.7	241 ± 3.8	125.0 ± 5.4
SFD	15.4 ± 0.90[a]	114 ± 3.3	107 ± 13.5[a]	−7.0 ± 10.8[a]
SFD-250	14.6 ± 1.50	111 ± 2.7	121 ± 2.8	10.0 ± 2.1
SFD-500	13.9 ± 2.30	109 ± 6.4	126 ± 0.7	17.0 ± 0.6
SFD-750	14.8 ± 2.20	117 ± 3.7	122 ± 3.9	5.0 ± 2.8
FFC	13.2 ± 1.35	115 ± 3.4	231 ± 9.7	116.0 ± 7.1
FFD	17.5 ± 0.28[b]	119 ± 3.8	132 ± 1.9[b]	13.0 ± 1.1
FFD-250	17.0 ± 2.00	120 ± 4.8	144 ± 7.0	24.0 ± 5.7
FFD-500	13.8 ± 0.21[b]	115 ± 4.3	155 ± 8.4[b]	40.0 ± 6.7[b]
FFD-750	14.4 ± 1.91	120 ± 5.6	146 ± 5.6	26.0 ± 4.8

SFC = Starch Fed control, SFD = Starch Fed Diabetic, FFC = Fibre Fed Control, FFD = Fiber Fed Diabetic. Groups with the numbers (eg. SFD–250) represent mg of butyric acid fed/day/kg body weight.

Values are mean ± SEM of 6 rats in control and 14 rats in diabetic groups.

[a] Statistically significant when compared to SFC at $p < 0.05$.

[b] Statistically significant when compared to SFD at $p < 0.05$.

Table 3
Butyric acid consumption and effect of butyric acid on water intake in control and diabetic rats

Groups	Water intake (ml/24h)	Butyric acid consumption (mg/kg body weight/day)
SFC	30.3 ± 4.1	—
SFD	120.0 ± 1.3[a]	—
SFD-250	100.0 ± 3.0	253.5 ± 13.4
SFD-500	92.2 ± 1.2[b]	492.6 ± 6.8
SFD-750	97.1 ± 2.8	723.1 ± 18.6
FFC	30.6 ± 1.5	—
FFD	110.9 ± 0.7[b]	—
FFD-250	84.7 ± 1.5	243.1 ± 4.7
FFD-500	77.1 ± 1.2[b]	486.0 ± 12.9
FFD-750	78.4 ± 0.9	756.3 ± 8.3

Abbreviations as in Table—2.

diabetic rats. Butyric acid feeding at 500 mg/kg body weight/day to starch-fed diabetic rats resulted in a lower dietary intake (13.9 ± 2.30 g/day). Diabetic rats fed with fiber and butyric acid at 500 mg/kg body weight/day showed a significant reduction in the amount of dietary intake (13.8 ± 0.21 g/day). In accordance with dietary intake, the control rats showed a steady increase in body weight. The gain in body weight in fiber-fed control rats (116.0 ± 7.1 g) was a little less compared to starch-fed control rats (125.0 ± 5.4 g). There was a marginal gain in body weight in fiber-fed diabetic rats (13.0 ± 1.1 g), whereas starch-fed diabetic rats lost weight (−7.0 ± 10.8 g) in spite of higher dietary consumption. Butyric acid feeding at 500 mg/kg body weight/day to diabetic animals showed a slight improvement in gain in body weight (15%). The fiber-fed diabetic group showed an improvement over the starch-fed diabetic group. Butyric acid feeding to the fiber-fed diabetic group showed a significant improvement in gain in body weight and was best at the 500 mg/kg body weight/day level.

3.2. Butyric acid consumption and effect of butyric acid on water intake in control and diabetic rats

Butyric acid consumption was measured daily (Table 3). The average consumption of butyric acid was around 250 (230–280), 500 (480–530) and 750 (720–780) mg/kg body weight/day level in the three groups reported SFD/FFD—250, SFD/FFD—500 and SFD/FFD—750, respectively.

Water intake in different groups was measured daily (Table 3). The starch and fiber fed control groups did not show much alteration in water intake over a period of 5 weeks (30 ml). The starch-fed diabetic group (120.0 ± 1.3 ml/24 h) showed a significant increase in water intake compared to control rats. The fiber-fed diabetic group consumed less water (110.9 ± 0.70 ml/24 h) compared to the starch-fed diabetic group. Feeding of butyric acid (500 mg/kg body weight/day level) to SFD resulted in a lower consumption of water (92.2 ml/24 h). On the other hand, butyric acid feeding (500 mg/kg body weight/day level) along with fiber showed a significant reduction in water intake (77.1 ± 1.2 ml/24 h).

3.3. Effect of butyric acid on urine volume, urine sugar and fasting blood sugar in control and diabetic rats

The excretion of urine was around 20 ml/24 h in control animals (Fig. 1). The urine output in the starch-fed diabetic group increased significantly with the progression of diabetic status to about 117 ml/24 h. Fiber feeding to diabetic rats showed a significant reduction in the volume of urine output (96 ml/24 h). Butyric acid feeding at 500 mg/kg body weight/day to the starch-fed diabetic group showed around a 10% improvement (108 ml/24 h) compared to the diabetic group. The fiber-fed diabetic group supplemented with butyric acid (500 mg/kg body weight/day level) excreted around 84 ml/24 h and was the most effective group.

There was an increased excretion of reducing sugar during diabetes (Fig. 2). Control rats excreted around 20 mg/24 h, while the diabetic animals excreted up to 4.0 g/24 h initially which increased significantly over a period of 5 weeks to around 7.2 g/24 h. The excretion of sugar in diabetic rats was significantly higher compared to control animals. Butyric acid feeding at 500 mg/kg body weight/day to the starch-fed diabetic group prevented the increase in excretion of urinary sugar (5.7 g/24 h) by 20%. The sugar excretion in the fiber-fed diabetic group did not show a significant increase over a period of time and was around 4.8 g/24 h at the end of the fifth week. This indicates that fiber in the diet minimized the increase in sugar levels and did not permit the diabetic status/diabetic nephropathic status to deteriorate further. Butyric acid feeding at 500 mg/kg body weight/day along with fiber showed a marked reduc-

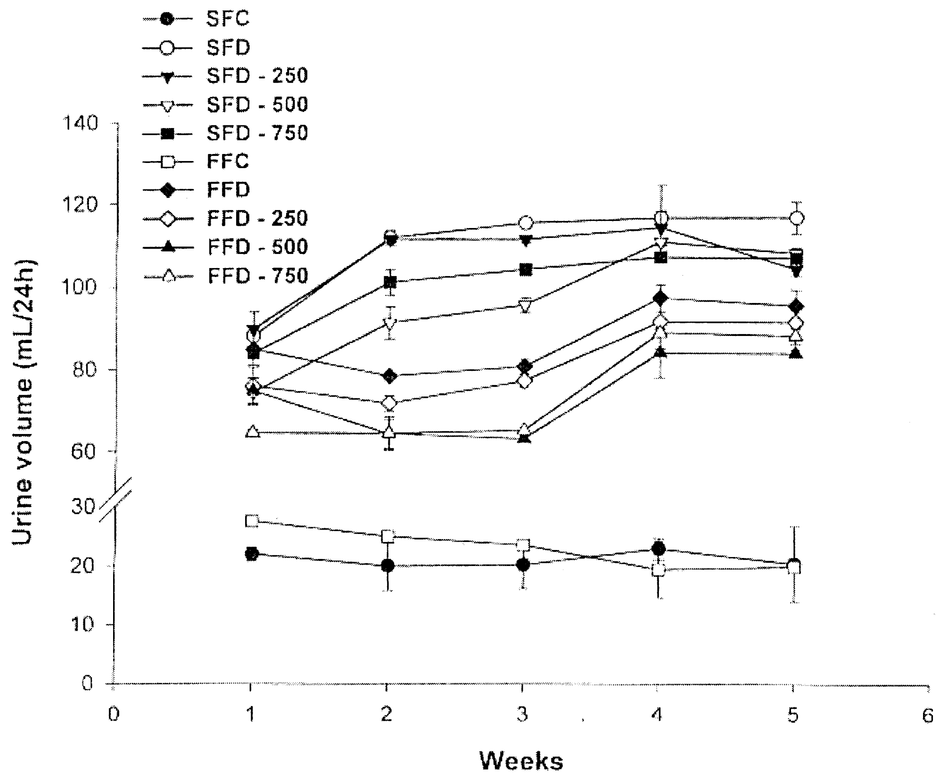

Fig. 1. Effect of butyric acid on urine output in control and diabetic rats. SFC—Starch Fed Control, SFD—Starch Fed Diabetic, FFC—Fiber Fed Control, FFD—Fiber Fed Diabetic. Groups with numbers (eg. SFD—250) represent mg of butyric acid fed/kg body weight/day.

tion (3.6 g/24 h) in sugar excretion (50%) compared to the starch-fed diabetic group (7.2 g/24 h).

Concentration of fasting blood glucose in various groups is given in Table 4. The control rats had fasting blood glucose levels around 105 mg/dl. The fasting blood glucose levels in the starch-fed diabetic rats significantly increased to around 270 mg/dl. Butyric acid feeding at 500 mg/kg body weight/day to the starch-fed diabetic group brought down the fasting blood glucose level (211 mg/dl) by 21%. The percentage of reduction was significant compared to the starch fed diabetic rats. Fiber feeding improved the fasting blood glucose level to 212 mg/dl. The increase of fasting blood glucose levels in the fiber-fed diabetic group was not as great as with the urinary sugar. Butyric acid feeding at 500 mg/kg body weight/day along with fiber showed a further reduction in fasting blood glucose level (180 mg/dl) 33%. This was significant compared to the starch-fed diabetic animals. This indicates that butyric acid in the diet improves the diabetic status not only from deteriorating further, but also has a beneficial effect in the management of the progression of the diabetic status.

4. Discussion

Diet and dietary fibers play significant roles in management of various diseases including diabetes [6,7]. The fermentation of dietary fibers to short chain fatty acids –butyric acid in particular, and the beneficial role on various physiological functions is of great importance [13,14]. The beneficial effect of dietary fibers on the diabetic status is well documented [28]. The results of the present study have shown that butyric acid feeding can further ameliorate the diabetic status, in terms of urine sugar, fasting blood sugar and various other parameters.

The diabetic animals lost body weight in spite of a high dietary consumption compared to control animals. The loss in weight was nearly 6% which improved significantly by 15% with butyric acid feeding over a period of time. Fiber in the diet and butyric acid feeding showed a significant improvement (50%) on gain in body weight. The levels of urinary sugar, an important criterion of the diabetic status showed a progressive increase every week compared to control rats. Excessive urine output (polyuria) and increased intake of water (polydipsia) were seen in the diabetic condition compared to control rats. Fiber in the diet alone showed a 10% reduction in urine output. Feeding of butyric acid at 500mg/kg body weight/day to fiber-fed diabetic rats showed a nearly 28% reduction. This was significant in terms of management of the diabetic status. The elevated levels of various metabolic parameters such as reducing sugar in urine and fasting blood sugar were significantly decreased in the presence of fiber and butyric acid. Butyric

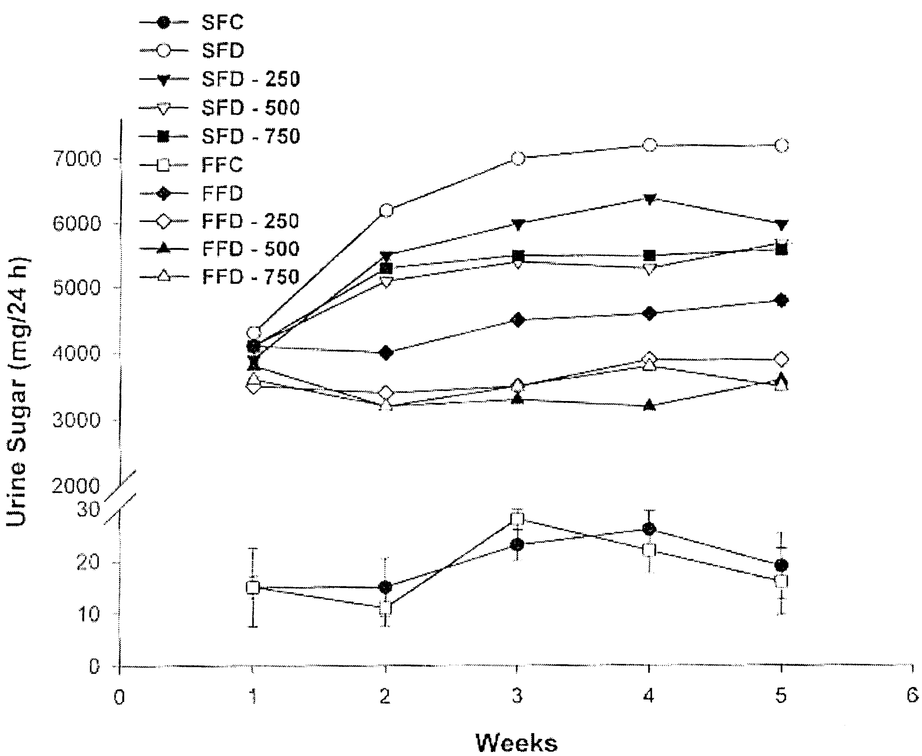

Fig. 2. Effect of butyric acid on urine sugar in control and diabetic rats. SFC—Starch Fed Control, SFD—Starch Fed Diabetic, FFC—Fiber Fed Control, FFD—Fiber Fed Diabetic. Groups with numbers (eg. SFD—250) represent mg of butyric acid fed/kg body weight/day.

acid feeding at 500 mg/kg body weight/day level was most effective in controlling the diabetic status.

The results showed that the diabetic rats fed with moderate levels of dietary fiber (FFD) faired comparably with or better than starch-fed diabetic animals that received 500–750 mg butyric acid/kg body weight/day (SFD 500–750). However, butyric acid feeding to the fiber-fed diabetic group (FFD–500) showed maximum beneficial effect. The threshold level of butyric acid was around 500 mg/kg body weight/day. The benefits observed in FFD–500 group are attributed to combined effect of, slow absorption of glucose and hence better glucose levels in the blood as a consequence of insloube matrix formed by dietary fiber in the intestine [8,9,29], slow release of butyric acid over a period of time by the fermentation of dietary fiber which in situ acts as reservoir of butyric acid [10,11,14,17], and supplementation of butyric acid. The results clearly demonstrated that butyric acid has beneficial role on diabetes and ameliorated urinary sugar, urinary volume and blood sugar levels besides various other parameters. These regimens may also be beneficial in preventing diabetic complications at the cellular and molecular levels. Studies in relation to the diabetic nephropathic state are under progress.

Table 4
Effect of butyric acid on fasting blood sugar in control and diabetic rats

Groups	Fasting blood sugar (mg/dl)		
	Initial	Fourth week	Fifth week
SFC	101.8 ± 11.1	105.4 ± 3.5	105.4 ± 1.2
SFD	217.5 ± 38.9[a]	258.3 ± 17.1[a]	269.5 ± 3.6[a]
SFD-250	212.3 ± 32.1	246.2 ± 20.2	248.5 ± 2.1
SFD-500	212.2 ± 18.4	216.3 ± 17.0	211.6 ± 1.7
SFD-750	214.0 ± 11.2	227.2 ± 4.2	217.9 ± 1.9
FFC	86.8 ± 11.0	106.0 ± 7.3	107.0 ± 0.4
FFD	208.4 ± 30.8	207.8 ± 14.6[b]	212.2 ± 4.7[b]
FFD-250	204.9 ± 24.7	205.0 ± 4.8	194.6 ± 0.4
FFD-500	201.2 ± 14.5	178.6 ± 13.7[b]	180.0 ± 1.3[b]
FFD-750	203.0 ± 18.7	187.3 ± 20.7	187.8 ± 2.3

Abbreviations as in Table—2.

Acknowledgments

Financial assistance of Department of Science and Technology (DST) –Health Sciences (SP/SO/B67/98) is gratefully acknowledged.

References

[1] Diabetes Mellitus–Report of WHO study group, WHO Techn Rep Ser 727 (1985) 1–113.
[2] D.H. Rohrbach, J.R. Hassel, H.K. Kleinman, G.R. Martin, Alterations in the basement membrane (heparan sulphate) proteoglycan in diabetic mice, Diabetes 31 (1982) 185–188.
[3] P.S. Mohan, W.G. Carter, R.G. Spiro, Occurrence of type VI collagen in extracellular matrix of renal glomeruli and its increase in diabetes, Diabetes 39 (1990) 31–37.

[4] H. Shimomura, R.G. Spiro, Studies on macromolecular components of human glomerular basement membrane and alterations in diabetes, Diabetes 36 (1987) 374–381.
[5] D.H. Rohrbach, G.R. Martin, Structure of basement membrane in normal and diabetic tissues, Ann N Y Acad Sci 401 (1982) 203–211.
[6] J.H. Cummings, H. Trowel, D. Burkitt, K. Heaton, IN: Dietary fibre, fibre depleted foods and diseases, Academic Press, London, 1985, pp. 161.
[7] G.A. Spiller, CRC Handbook of Dietary Fibre In Human Nutrition, CRC Press Inc., Florida, 1986.
[8] F.Q. Nuttall, Dietary fibre in the management of diabetes, Diabetes 42 (1993) 503–508.
[9] N.K. Mujumdar, High fibre diet in diabetes mellitus, J Indian Med Assoc 93 (1995) 117–119.
[10] J.H. Cummings, W.J. Branch, in: Vahouny, G.V. Kritchevsky (Eds.), Dietary fibre: basic and clinical aspects, Plenum Press, New York, 1986, pp. 131–149.
[11] L.D. Bourquin, E.C. Titgemeyer, K.A. Garleb, G.C. Fahey, Short-chain fatty acid production and fiber degradation by human colonic bacteria: effects of substrate and cell wall fraction procedures, J Nutr 122 (1992) 1508–1520.
[12] C.A. Edwards, in: D. Kritchevsky, C. Bonfield, (Eds.), Dietary fibre in health and disease, Eagan Press, Minnesota, USA, 1995, pp. 58–71.
[13] L.D. Bourquin, E.C. Titgemeyer, G.C. Fahey, Fermentation of various dietary fibre sources by human fecal bacteria, Nutr Res 16 (1996) 1119–1131.
[14] J.H. Cummings, G.T. Macfarlane, The control and consequences of bacterial fermentation in the human colon, J Appl Bacteriol 70 (1991) 443–459.
[15] M.G. Riggs, R.G. Whittaker, J.R. Neumann, V.M, Ingram, n-Butyrate causes histone modification in HeLa and friend erythroleukaemia cells, Nature 268 (1977) 462–463.
[16] C. Calabresse, L. Venturini, G. Ronco, P. Villa, C. Chomienne, D. Belpomme, Butyric acid and its monosaccharide ester induce apoptosis in the HL-60 cell line, Biochem Biophys Res Commun 195 (1993) 31–38.
[17] J.G. Smith, W.H. Yokoyama, J.B. German, Butyric acid from the diet: actions at the level of gene expression, Crit Rev Food Sci Nutr 38 (1998) 259–297.
[18] K.G. Jacobsson, J. Riesenfeld, V. Lindaw, Biosynthesis of heparin—effects of n-butyrate on cultured mast cells, J Biol Chem 260 (1985) 12154–12159.
[19] S. Shah, P. Lance, T.J. Smith, C.S. Berenson, S.A. Cohen, P.J. Horvath. J.T.Y. Lau, H. Bauman, n-Butyrate reduces the expression of β-galactoside α2, 6-sialyltransferase in HepG2 cells, J Biol Chem 267 (1992) 10652–10658.
[20] T. Ohta, N. Furukawa, F. Yonemori, K. Wakitani, JTT-608 controls blood glucose by enhancement of glucose stimulated insulin secretion in normal and diabetic mellitus rats, Eur J Pharmacol 367 (1999) 91–99.
[21] A.E. Karlsent, W.Y. Fugimoto, P. Rabinovitch, S. Dube, A. Lernmark, Effects of sodium butyrate on proliferation dependent insulin gene expression and insulin release in glucose sensitive RIN-5AH cells, J Biol Chem 266 (1991) 7542–7548.
[22] V. Della Pietra, A. Borreiello, A. Oliva, S. Indaco, T. Yamamoto, V. Zappia, Genes modulated by histone acetylation as new effectors of butyrate activity, FEBS Lett 499 (2001) 199–204.
[23] J.G. Bieri, G.S. Stoewsand, G.M. Briggs, R.W. Phillips, J.C. Woolard, J.J. Knapka, Report of the American Institute of Nutrition Ad Hoc Committee on Standards for Nutritional Studies, J Nutr 107 (1977) 1340–1348.
[24] G.M. Hatch, S.G. Cao, A. Angel, Decrease in cardiac phosphatidylglycerol in streptozotocin induced diabetic rats does not affect cardiolipin biosynthesis: evidence for distant pools of phosphatidyl glycerol in the heart, Biochem J 306 (1995) 759–764.
[25] A.S.G. Hugget, D.A. Nixon, Use of glucose oxidase, peroxidase and O-dianisidine in the determination of blood glucose and urinary glucose, Lancet 273 (1957) 366–370.
[26] G.L. Miller, Use of dinitrosalicylic acid reagent for determination of reducing sugar, Anal Chem 31 (1959) 426–428.
[27] G.W. Snedecor, W.G. Cochran, in: Statistical methods, 6th ed., Ames, Iowa State Univ Press, 1976, pp. 298.
[28] C. D. Nandini, K. Sambaiah, P.V. Salimath, Effect of dietary fibre on intestinal and renal disaccharidases in diabetic rats, Nutr Res 20 (2000) 1301–1307.
[29] S. P. Plaami, Content of dietary fibre in food and its physiological effects, Food Rev Intern 13 (1997) 29–76.