Journal of Chromatography B, 879 (2011) 3331–3336

Contents lists available at ScienceDirect

Journal of Chromatography B

journal homepage: www.elsevier.com/locate/chromb

Enantioselective determination of 3-hydroxybutyrate in the tissues of normal and streptozotocin-induced diabetic rats of different ages☆

Wei-Yu Hsu[a], Chen-Yi Kuo[b,1], Takeshi Fukushima[c], Kazuhiro Imai[d], Chien-Ming Chen[e], Pen-Yuan Lin[a], Jen-Ai Lee[a,*]

[a] School of Pharmacy, College of Pharmacy, Taipei Medical University, No. 250 Wuxing St., Taipei 11031, Taiwan
[b] Department of Surgery, Chi-Mei Hospital, No. 901, Chung-Hwa Rd., Yong Kang City, Tainan 710, Taiwan
[c] Faculty of Pharmaceutical Sciences, Toho University, 2-2-1 Miyama, Funabashi-shi, Chiba 274-8510, Japan
[d] Research Institute of Pharmaceutical Sciences, Musashino University, 1-1-20 Shinmachi, Nishitokyo-shi, Tokyo 202-8585, Japan
[e] Department of Electro-Optical Engineering, National Taipei University of Technology, No. 1, Sec. 3, Chung-Hsiao E. Rd., Taipei 106, Taiwan

ARTICLE INFO

Article history:
Received 29 November 2010
Accepted 27 July 2011
Available online 4 August 2011

Keywords:
D-3-Hydroxybutyrate
L-3-Hydroxybutyrate
Enantiomer
Column-switching HPLC
Diabetes

ABSTRACT

L-3-Hydroxybutyrate (3HB) and D-3HB are enantiomers that exist in various rat tissues, and the ratio of the 2 compounds is of importance since it may affect glucose utilization in cardiomyocytes. In this study, we determined the concentrations of L-3HB and D-3HB in the tissues of normal and streptozotocin (STZ)-induced diabetic rats of different ages by column-switching high-performance liquid chromatography using a fluorescence detection system. In normal rats, the levels of L-3HB peaked at 8 weeks of age in the cerebrum, liver, spleen, lung, kidney, adrenal gland, and heart and then decreased afterwards. The concentrations of L-3HB were the highest in the heart, with 26.24 ± 13.74 μmol/mg protein. In addition, there was an increase in the levels of (D + L)-3HB, D-3HB, and L-3HB in the tissues of diabetic rats with time, whereas the ratios of L-3HB to (D + L)-3HB declined (46.44% vs. 21.03%, $P < 0.05$, in heart tissue after 24 weeks of STZ treatment). Both the concentration and the ratio of L-3HB may be associated with disease conditions, and the determination of L-3HB may help clarify the role of L-3HB under physiological and pathological conditions.

1. Introduction

D-3-Hydroxybutyrate (D-3HB) and L-3-hydroxybutyrate (L-3HB), enantiomers of the chiral hydroxy acid, exist in various mammalian tissues. Since D-3HB is the major enantiomer in the tissues of human beings and the main component of ketone bodies, its biosynthesis and the metabolic pathways responsible for its production have been investigated in detail [1]. D-3HB, acetone, and acetoacetate are components of ketone bodies, and thereby function as effective precursors for the biosynthesis of fatty acids and sterols in the early developmental stages of the central nervous system (CNS) in rats. In the first 9–11 days in the life of a rat, the brain, spinal cord, skin, kidney, and liver utilize acetoacetate and D,L-3HB to synthesize fatty acids [2]. Although L-3HB is more favorable than other ketone bodies for the synthesis of sterols and fatty acids in the brain, spinal cord, and kidney [3], the oxidation rate of L-3HB has been found to be lower than that of D-3HB [4].

In the heart, D-3HB and L-3HB have different effects on the transient K^+ outward current (I_{to}) [5]. D-3HB has no effect on I_{to}, whereas L-3HB causes it to decrease, such that repolarization defects may occur in myocardial cells and result in cardiac abnormalities in patients with diabetes [5]. Moreover, L-3HB is able to block I_{to} in rat heart cells, but D-3HB does not have this effect. In addition, it has been reported that equal concentrations of D-3HB and L-3HB, which were predicted to have an inhibitory effect, showed no effect on the blockage of I_{to} in rat myocardial cells [6].

Since these different actions have been reported for D-3HB and L-3HB, we recently developed a column-switching high-performance liquid chromatography (HPLC) method to determine the concentrations of D-3HB and L-3HB in mammalian tissues [7,8]. To better understand the physiological role of the minor enantiomer, L-3HB, in maturation and development, we employed rats of different ages to observe age-dependent alterations, because postnatal changes in the utilization of ketone bodies or glucose have been reported [9].

We have previously reported that D- and L-3HB were detected in myocardial cell lines; D-3HB had an inhibitory effect, while L-3HB

Abbreviations: D-3HB, D-3-hydroxybutyrate; L-3HB, L-3-hydroxybutyrate; NBD-PZ, 4-nitro-7-piperazino-2,1,3-benzoxadiazole; STZ, streptozotocin.

☆ This paper is part of the special issue "Analysis and Biological Relevance of D-Amino Acids and Related Compounds", Kenji Hamase (Guest Editor).

* Corresponding author. Tel.: +886 2 2736 1661x6125; fax: +886 2 2736 1661x6120.
E-mail address: jenai@tmu.edu.tw (J.-A. Lee).

[1] Co-first author: Chen-Yi Kuo.

1570-0232/$ – see front matter © 2011 Elsevier B.V. All rights reserved.
doi:10.1016/j.jchromb.2011.07.038

had the opposite effect on glucose utilization [8]. In addition to our previous *in vitro* study [8], the present study employed an *in vivo* disease model of diabetes, streptozotocin (STZ)-induced diabetic rats, to determine the concentration of D- and L-3HB in various rat tissues in normal and diabetic rats at different ages using our established HPLC method.

2. Experimental

2.1. Materials

2.1.1. Chemicals

Sodium D-3HB and L-3HB were purchased from Wako Pure Chemicals (Osaka, Japan); triphenylphosphine (TPP), 2,2′-dipyridyl disulfide (DPDS), and 4-nitro-7-piperazino-2,1,3-benzoxadiazole (NBD-PZ), from Tokyo Chemical Industry (Tokyo, Japan); trifluoroacetic acid (TFA), from Riedel-de Haën (Seelze, Germany); propionic acid, from Nacalai Tesque (Kyoto, Japan); HPLC-grade acetonitrile (MeCN) and methanol (MeOH), from Merck (Darmstadt, Germany); Biosil ODS column (150 mm × 4.6 mm i.d.; 5 μm); and Chiralcel OD-RH (OD-RH) (150 mm × 4.6 mm i.d.; 5 μm), from Biosil Chemical (Taipei, Taiwan) and Daicel Chemical Industries (Osaka, Japan).

2.1.2. Animals

Male Sprague-Dawley rats (National Laboratory Animal Breeding and Research Center, Taipei, Taiwan) were housed in an environmentally controlled room at 25 °C with a 12-h light/12-h dark cycle. The animal experiments were approved by the Animal Care and Use Committee of Taipei Medical University, Taiwan (approval number: LAC940079). The tissues of normal rats at 4, 8, 16, and 24 weeks of age were resected and homogenized as described below. To induce diabetes, 7-week-old rats were intraperitoneally administered streptozotocin (STZ) (80 mg/kg body weight) (Sigma, St. Louis, MO, USA). Rats receiving the same volume of citrate buffer were used as controls. The tissues of normal and diabetic rats (4 rats per group) were resected and homogenized after 1, 4, 12, and 24 weeks of administration.

2.2. Methods

2.2.1. Enantiomeric analysis of D- and L-3HB in rat homogenates

Rats were dissected to allow the collection of blood from the abdominal aorta. Different tissues were then collected and homogenized with ice-cold PBS buffer (0.08 M Na_2HPO_4, 0.02 M KH_2PO_4, and 0.145 M NaCl). The concentration of D- and L-3HB in the homogenates was then analyzed according to the method described in our previously published articles [7,8]. Briefly, the samples were prepared as follows: a 10 μL aliquot of 1 mM internal standard propionic acid was added to 50 μL of the homogenate. For deproteinization, the suspension was then brought to 200 μL with ethanol. The solution was mixed and centrifuged at 700 × *g* for 5 min at 4 °C. For derivatization, 50 μL of the supernatant was added to 50 μL of 2 mM NBD-PZ, and then, 50 μL each of 280 mM TPP and DPDS were added to each sample and mixed. The mixture was allowed to stand for 3 h at 30 °C before 100 μL of 0.1% TFA in H_2O was added to terminate the reaction. The resulting solution was injected into a column-switching HPLC system. The total 3HB in the homogenates was isolated and quantified using a Biosil ODS column (150 mm × 4.6 mm i.d., 5 μm) and a mobile phase of methanol/H_2O (33/67, v/v) at a flow rate of 0.7 mL/min. To confirm the identity of the peak representing 3-HB, selected samples were treated with D-3HB dehydrogenase, which catalyzes the conversion of D-3HB to acetoacetate and causes a significant reduction or disappearance of the 3-HB peak, to confirm its identity as the correct peak. The enantiomeric separation of 3HB was achieved by elution with acetonitrile/H_2O (34/66, v/v) using tandem OD-RH columns (300 mm total length × 4.6 mm i.d.) (Daicel Co., Osaka, Japan). Fluorescence detection was performed at 547 nm with a 491 nm excitation wavelength. D- and L-3HB concentrations were quantitatively determined from the peak areas on the chromatograms. In addition to comparing the retention times, D-3HB dehydrogenase was used to further confirm the peak that represented total (D + L)-3HB. Enzymes specific for L-3HB are extremely difficult to obtain and are not commercially available. However, D-3HB dehydrogenase not only catalyzes D-3HB to generate acetoacetate but also cross-reacts with L-3HB [10]. As a result, adding D-3HB dehydrogenase resulted in disappearance of the peak for both enantiomers, and this reaction can be used to confirm that the peaks are 3HB enantiomers, not other contaminants. The structures of 3HB and the fluorescent derivatization reagent NBD-PZ are described in Fig. 1, which indicates the derivatization scheme of 3HB with NBD-PZ.

2.2.2. Determination of protein levels

The supernatants of the tissue homogenates were collected, and the total protein concentrations were quantified by the Bradford method [11] with Bio-Rad protein dye (Bio-Rad Laboratories, Hercules, CA, USA). Bovine serum albumin was used as the protein standard.

2.3. Statistical analysis

Data for L-3HB in rat tissues are expressed as mean ± standard error (SE). The statistical significance of differences between L-3HB content in normal rat tissue at different weeks of age was analyzed by one-way ANOVA with Tukey's tests for post hoc comparisons. The statistical significance of differences in L-3HB content between normal and diabetic rat tissues groups was analyzed by the independent two-sample *t*-test. Differences were considered significant when a $P < 0.05$ was obtained.

3. Results

3.1. L-3HB content and percentage in normal rat tissues at different weeks of age

In this study, (D + L)-3HB in the various tissues was derivatized with the fluorescence reagent NBD-PZ. The derivatives were then separated from the other components on an ODS column, followed by enantiomeric separation performed according to our previous column-switching HPLC method using fluorescence detection [7,8].

L-3HB was detected in the heart (Fig. 2A), as well as in the serum, cerebrum, cerebellum, testis, liver, spleen, lung, kidney, and adrenal gland of 4-, 8-, 16-, and 24-week-old rats (Fig. 2A). The level of L-3HB in heart tissues was the highest in rats at 8 weeks of age (26.24 ± 13.74 μmol/mg protein; Fig. 2A). Moreover, in 8-week-old rats, the concentration of total (D + L)-3HB in heart tissues was 74.72 ± 32.77 μmol/mg protein, nearly 2.5 times higher than that in any other tissue (data not shown).

L-3HB levels reached a peak in rats at 8 weeks of age in the cerebrum, testis, liver, spleen, lung, kidney, and adrenal gland and thereafter decreased (Fig. 2A). L-3HB concentrations were significantly higher ($P < 0.05$) in the cerebellum of rats at 4 weeks of age (1.84 ± 0.25 μmol/mg protein) than those at 8, 16, or 24 weeks (0.67 ± 0.25 μmol/mg protein; 0.34 ± 0.09 μmol/mg protein; 1.00 ± 0.08 μmol/mg protein, respectively; Fig. 2A).

Fig. 2B shows the contribution of L-3HB relative to total (D + L)-3HB from various tissues in normal rats. The highest relative levels of L-3HB were observed in the adrenal gland at 8 weeks of age (56.90%), and the levels declined gradually at 16 (45.10%) and 24 weeks of age (24%; significantly lower than at 8 weeks of age;

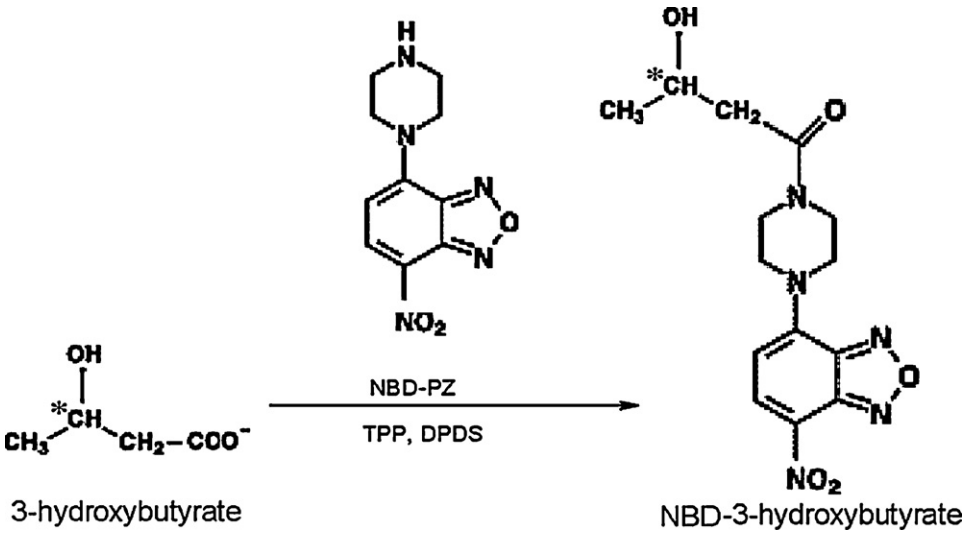

Fig. 1. Chemical structures of 3-hydroxybutyrate, the fluorescent reagent 4-nitro-7-piperazino-2,1,3-benzoxadiazole (NBD-PZ), and the reaction scheme producing NBD-3-hydroxybutyrate. *Indicates a chiral carbon.

$P < 0.05$). A chromatogram of 3-HB in the rat adrenal gland at 16 weeks is shown in Fig. 3, and the enantiomeric separation of D- and L-3HB in the 16-week-old rat adrenal tissue is also shown in Fig. 3.

As shown in Fig. 2B, a much smaller contribution of L-3HB to total 3HB was observed in the testis, but a similar age-related decline in the L-3HB fraction was observed, with significantly lower levels at 16 and 24 weeks of age ($5.28 \pm 0.56\%$ and $4.66 \pm 0.70\%$, respectively) compared to that at 4 weeks of age ($7.98 \pm 1.03\%$). The relative levels of L-3HB in heart tissues were high at week 8 (31.64%) and were maintained at weeks 16 (28.44%) and 24 (35.34%), all of which were significantly higher than that in heart tissues from 4-week-old rats ($P < 0.05$). A similar pattern was seen in the kidney, in which the contribution of L-3HB at 8 and 24 weeks of age were significantly higher than that at 4 weeks of age. In the spleen, the percent-

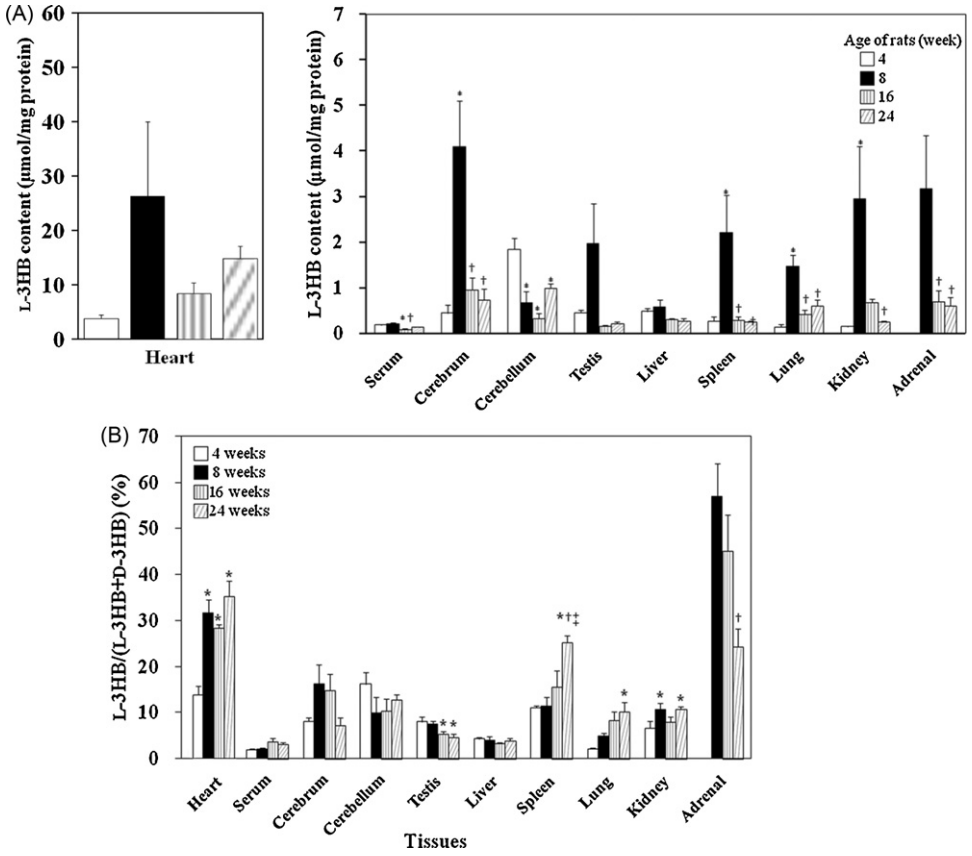

Fig. 2. Concentration and percentage of L-3HB in the tissues of rats at 4, 8, 16, and 24 weeks of age. (A) L-3HB concentrations in the heart, serum, cerebrum, cerebellum, testis, liver, spleen, lung, kidney, and adrenal gland. (B) Percentages of L-3HB/(D + L)-3HB in the tissues. Values are represented as means with standard deviation. Ratio of L-3HB (μM) to protein (mg) in the tissues. *$P < 0.05$ for rats at 4 weeks of age. †$P < 0.05$ as compared with rats at 8 weeks of age. ‡$P < 0.05$ for rats compared at 16 weeks of age ($n = 4$).

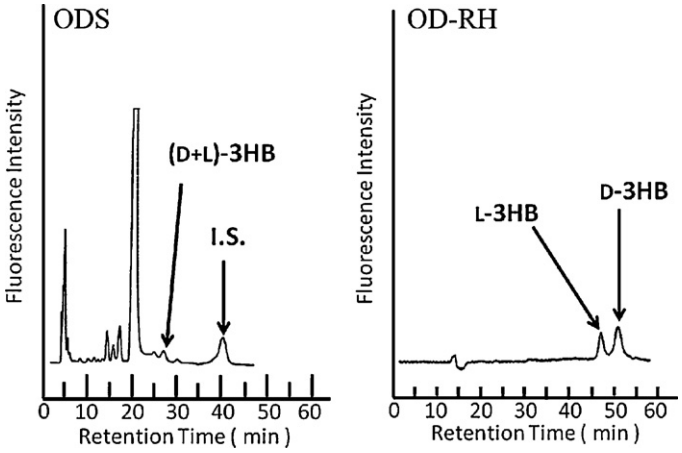

Fig. 3. Chromatograms of D,L-3HB in the rat adrenal gland at 16 weeks of age. I.S. = internal standard (propionic acid). ODS: Adrenal gland homogenate derivatized with NBD-PZ was eluted by the Biosil ODS column; chromatogram was from the first integrator. Retention times of the total (D + L)-3HB derivatives and I.S. were about 27 and 40 min, respectively. OD-RH: Enantiomeric separation of D- and L-3HB derivatives isolated from the rat adrenal tissue. The Chiralcel OD-RH was selected as the chiral column to perform an efficient separation, and the chromatogram was from the second integrator.

age of L-3HB increased with age and was significantly higher at 24 weeks of age (25.15 ± 1.61%) than levels at 4, 8, and 16 weeks of age (11.05 ± 0.45%, 11.38 ± 1.89%, 15.56 ± 3.47%, respectively). Although the percentage of L-3HB tended to increase with age in the lung, only the percentage at 24 weeks was significantly higher than that at 4 weeks of age (10.09 ± 2.20% vs. 2.09 ± 0.26%) (Fig. 2B).

3.2. L-3HB content in the serum, kidney, urine, and heart of STZ-induced diabetic rats

Body weight and blood glucose levels of rats at 0 and 4 days after 1, 4, 12, and 24 weeks following STZ administration are shown in Table 1. As compared to citrate buffer-injected age-matched controls, STZ-injected rats showed significant decrease in the body weight 4 days after induction and significant increase in blood glucose levels (greater than 400 mg/dL), indicating that diabetes was successfully induced in these rats.

Serum and urine are the most easily tested samples in clinical practice. Since diabetes can induce complications such as diabetic nephropathy and cardiovascular diseases, measuring changes in D- and L-HB levels in the kidney and heart could provide further insights into the diabetic pathophysiology. In addition, the amount and ratio of D- and L-HB in the heart is related to glucose utilization. As such, serum, urine, heart, and kidney samples were selected as the key tissues to be tested in this study.

Chromatograms of (D + L)-3HB separation for the serum of normal and diabetic rats 4 weeks after STZ administration (the 11th week of age) are shown in Fig. 4. Chromatograms of the enantiomeric separation of D- and L-3HB are also shown in Fig. 4.

As shown in Fig. 5A, the concentration of L-3HB was higher in the tissues of diabetic rats than in the tissues of normal rats (Fig. 5A), except in the heart tissue after 1, 12, and 24 weeks of STZ administration (Fig. 5A). Among all tissues, the level of L-3HB was the highest in the urine of diabetic rats after 4 weeks of STZ treatment (137.26 ± 65.35 μM; Fig. 5A). Significant differences in L-3HB concentrations were observed between the sera of diabetic and control rats at 4 and 12 weeks after STZ administration (4 weeks: 1.37 μM for normal rats vs. 14.61 μM for diabetic rats; 12 weeks: 1.59 μM for normal rats vs.8.71 μM for DM rats; Fig. 5A).

As shown in Fig. 5A, the concentration of L-3HB in the urine of diabetic rats increased from week 1 to 4 after STZ treatment (2.28 ± 0.86 μM to 137.26 ± 65.35 μM) and then decreased from week 12 and 24.

3.3. Percentage of L-3HB in the serum, kidney, urine, and heart of STZ-induced diabetic rats

Although the (D + L)-3HB, D-3HB, and L-3HB content in the tissues of diabetic rats tended to increase over the weeks following STZ injection, the ratios of L-3HB to (D + L)-3HB decreased relative to those of normal rats (Fig. 5B). This decrease may be the result of a much greater increase in the D-3HB concentration in the diabetic rats. In heart tissues from control rats, the relative concentration of L-3HB increased with age, from 14.96% at week 1 to 46.44% at week 24. The percentages of L-3HB were higher in normal rats at all time points than those in the diabetic rats, with significant differences observed at 4, 12, and 24 weeks after STZ treatment. Heart tissues from diabetic rats showed a steady decline in relative L-3HB levels from 1 week (11.60%) to 12 weeks (4.96%) after STZ treatment, but the percentage of L-3HB increased 24 weeks after STZ treatment to 21.03%, which was still significantly lower than that for the corresponding control rats.

Similar patterns were also observed for the change in the percentage of L-3HB in the kidney and urine for both normal and diabetic rats. However, after 1 week of STZ treatment, the percentage of L-3HB in the kidney and urine were higher in diabetic rats than in control rats (5.21% vs. 3.73% and 11.14% vs. 6.16%, respectively). At later time points, however, the relative levels of L-3HB in diabetic rats were lower than those in control rats. In particular, the percentage of L-3HB was significantly lower in the urine of diabetic rats 4 and 24 weeks after STZ injection. The percentage of L-3HB in serum from diabetic rats was the highest 24 weeks after STZ injection (3.11%). At week 12, the percentage of L-3HB in serum from diabetic rats was significantly lower than that in controls rats (2.41% vs. 1.06%) (Fig. 5B).

4. Discussion

The aim of this study was to investigate the physiological significance of the minor 3-HB enantiomer, L-3HB by examining age-related changes in its concentration in brain and peripheral tissues, as well as serum and urine. Furthermore, since diabetic stage

Table 1
Body weight and blood glucose data of normal and diabetic rats (DM).

Time after treatment	Body weight (g)		Blood glucose (mg/dL)	
	Normal	DM	Normal	DM
0 day	239.73 ± 14.11	246.39 ± 10.58	131.20 ± 24.03	125.17 ± 20.30
4 days	273.07 ± 13.59	248.83 ± 12.90*	122.20 ± 23.43	466.00 ± 80.53*
1 week	278.00 ± 8.38	257.25 ± 9.44*	243.75 ± 58.22	485.50 ± 106.91*
4 weeks	412.13 ± 14.76	267.75 ± 30.36*	200.00 ± 39.23	495.50 ± 115.79*
12 weeks	561.05 ± 47.14	324.05 ± 35.58*	124.00 ± 55.90	489.50 ± 80.27*
24 weeks	572.83 ± 22.48	363.70 ± 32.33*	158.67 ± 26.10	483.60 ± 76.15*

* $P < 0.05$, compared with normal rats ($n = 4$).

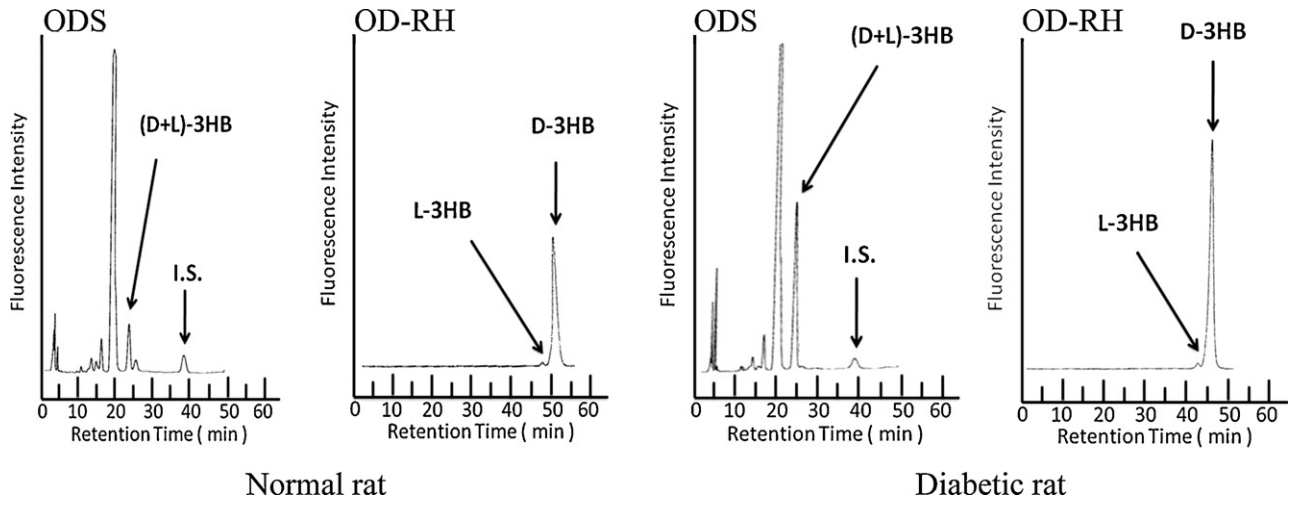

Fig. 4. Chromatograms of D,L-3HB in the serum of normal and diabetic rats after 4 weeks of citrate buffer (vehicle) or STZ treatment. I.S. = internal standard (propionic acid). Normal rat ODS: Normal rat serum derivatized with NBD-PZ was eluted by the Biosil ODS column. Normal rat OD-RH: Enantiomeric separation of D- and L-3HB derivatives isolated from normal rat serum. Diabetic rat ODS: Chromatogram of D,L-3HB derivatives in diabetic rat serum. Diabetic rat OD-RH: Enantiomeric separation of D- and L-3HB derivatives isolated from diabetic rat serum.

may alter the relative concentrations of L- and D-3HB in rats [8], we also aimed to assess changes in the relative concentrations of these 2 enantiomers in parallel with the development of STZ-induced diabetes in rats.

The present data show a detailed distribution of D- and L-3HB in rats of 4–24 weeks age. L-3HB was detected in most tissues of 4-week-old rats, and relatively higher amounts were found in the tissues of 8-week-old rats (Fig. 2A). L-3HB levels in rats at 8 weeks of age were highly variable. These data may suggest that 8 weeks represents a critical transition period between adolescent and adult rats and that some rats reach adulthood before others. Among all the organs and tissues studied, endogenous L-3HB showed the highest content in the heart (Fig. 2A). Heart tissue acquires more than 80% of its total energy requirements from ATP generated from the oxidation of fatty acids [12]. The other 20% of the total energy may be supplied by glucose. Furthermore, the need for glucose by the heart tissue is higher than that of other organs [13], which may explain why the highest L-3HB content is found in the heart tissue. We had previously found [8] that D-3HB inhibited the myocardial utilization of glucose, which could be restored after addition of L-3HB into the medium. Our current findings suggest a possible role for endogenous L-3HB in regulating the balance between the use of glucose or ketone bodies in cardiac cells. These data suggest that L-3HB may play a physiological role in the regulation of cardiac function by modifying transient K^+ outward currents [6].

It is known that (D + L)-3HB plays an important role in the development of brain tissue [2]. Because (D + L)-3HB is a source of energy in the brain of newborn rats, it has also been hypothesized that (D + L)-3HB might be a crucial constituent in the development of the neuronal system [14]. Under certain circumstances such as starvation or diabetes, D-3HB is used as the energy source, since the brain cannot use glucose effectively [15]. Our present study revealed a higher L-3HB content in the cerebellum of young rats compared to adult ones (Fig. 2A), which suggests that L-3HB may also be associated with the development of the cerebellum. Our data may indicate that both the L- and the D-3HB play important roles in

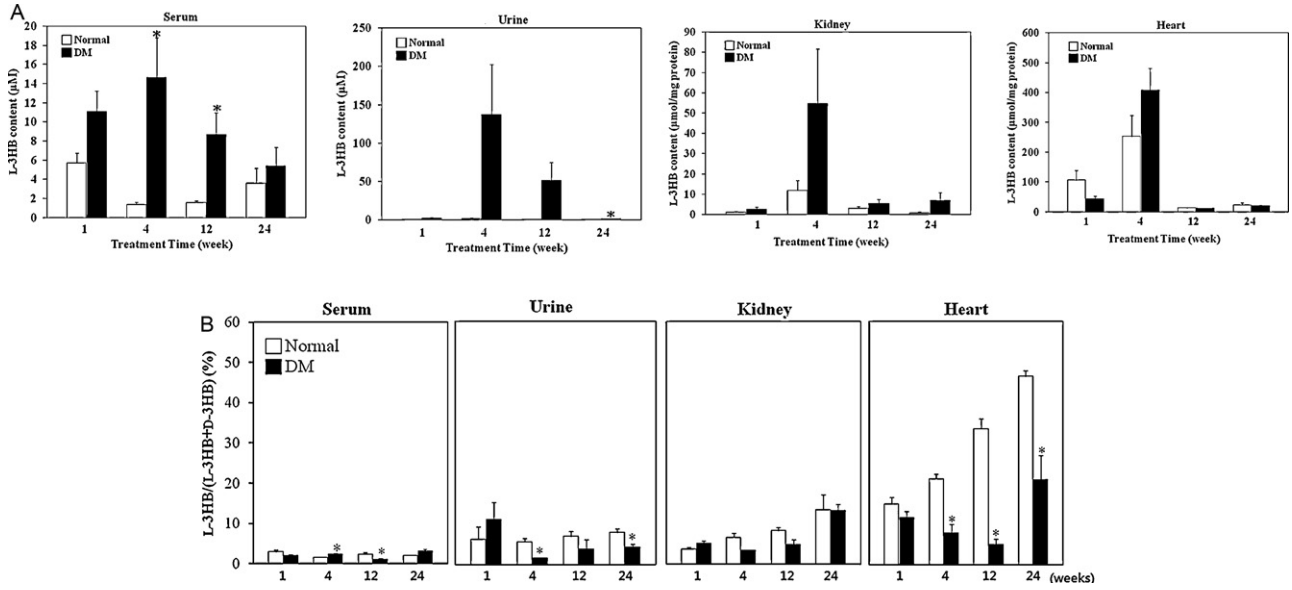

Fig. 5. Concentration and percentage of L-3HB in the tissues of normal and diabetic rats after 1, 4, 12, and 24 weeks of citrate buffer (vehicle) or STZ treatment. (A) L-3HB concentrations in the serum, urine, kidney, and heart (B) percentages of L-3HB/(D + L)-3HB in the tissues. *$P < 0.05$ vs. normal rats ($n = 4$).

neuronal development. Furthermore, L-3HB may be important at a critical stage of development that occurs with a different time course in different brain regions.

With respect to the hydroxybutyrate in diabetic rats induced by STZ, Reusch and colleagues reported that polyhydroxybutyrate (PHB) content in tissues other than the brain after 3 weeks showed 3- to 8-fold increase at this time point [16]. Therefore, they speculated that complications from diabetes may increase the PHB content, and therefore suggested that blood PHB may serve as a disease marker. Similarly, significant increases (5- to 10-fold) in serum L-3HB content were observed at 4 and 12 weeks in STZ-induced diabetic rats (Fig. 5A); therefore, L-3HB may also be a useful marker for diabetes. L-3HB contents in heart and kidney were significantly increased in diabetic rats (Fig. 5A). Further research is needed to determine the association of L-3HB in heart and kidney with specific disease conditions.

5. Conclusion

We have reported for the first time alterations in both D- and L-3HB content in various tissues of normal and diabetic rats of different ages by using our column-switching HPLC with fluorescence detection. Most tissues of 8-week-old rats had relatively higher levels of L-3HB compared to those in younger rats, with the highest concentration found in heart tissues. L-3HB content increased significantly in the diabetic stages compared to most tissues under normal conditions, but the percentage of L-3HB decreased significantly in the diabetic stages. Our results suggest that alterations in the concentration and percentage of L-3HB in tissues under disease conditions may warrant further research.

Acknowledgments

This work was financially supported by the Chi-Mei Medical Center (95CM-TMU-16).

References

[1] A.L. Lehninger, D.L. Nelson, M.M. Cox, Principles of Biochemistry, 2nd ed., Worth, New York, 1993.
[2] J. Edmond, J. Biol. Chem. 249 (1974) 72.
[3] R.J. Webber, J. Edmond, J. Biol. Chem. 252 (1977) 5222.
[4] K.R. Swiatek, G.J. Dombrowski Jr., K.L. Chao, Biochem. Med. 31 (1984) 332.
[5] C. Hipolito-Reis, E. Bailey, W. Bartley, Int. J. Biochem. 5 (1974) 31.
[6] B. Doepner, S. Thierfelder, H. Hirche, K. Benndorf, J. Physiol. 500 (1997) 85.
[7] Y.C. Tsai, T.H. Liao, J.A. Lee, Anal. Biochem. 319 (2003) 34.
[8] Y.C. Tsai, Y.C. Chou, A.B. Wu, C.M. Hu, C.Y. Chen, F.A. Chen, J.A. Lee, Life Sci. 78 (2006) 1385.
[9] W.D. Lust, S. Pundik, J. Zechel, Y. Zhou, M. Buczek, W.R. Selman, Metab. Brain Dis. 18 (2003) 195.
[10] D.H. Williamson, P. Kunenzel, Biochem. J. 121 (1971) 571.
[11] M.M. Bradford, Anal. Biochem. 72 (1976) 248.
[12] R.W. Brownsey, A.N. Boone, M.F. Allard, Cardiovasc. Res. 34 (1997) 3.
[13] A. Zorzano, L. Sevilla, M. Camps, C. Becker, J. Meyer, H. Kammermeier, P. Munoz, A. Guma, X. Testar, M. Palacin, J. Blasi, Y. Fischer, Am. J. Cardiol. 80 (1997) 65A.
[14] T. Arakawa, T. Goto, Y. Okada, Neurosci. Lett. 130 (1991) 53.
[15] J. Edmond, Canadian Journal of Physio, Pharma 70 (Suppl.) (1992) S118.
[16] R.N. Reusch, E.M. Bryant, D.N. Henry, Acta Diabetol. 40 (2003) 91.