Biochemical Pharmacology, Vol. 52, pp. 247–252, 1996.

ELSEVIER

ISSN 0006-2952/96/$15.00 + 0.00
PII S0006-2952(96)00201-8

NAD^+ Biosynthesis and Metabolic Fluxes of Tryptophan in Hepatocytes Isolated from Rats Fed a Clofibrate-Containing Diet

*Mariko Shin, Yoshimi Mori, Aki Kimura, Yukari Fujita, Kuniko Yoshida, Keiji Sano and Chisae Umezawa**

SCHOOL OF PHARMACY, KOBE GAKUIN UNIVERSITY, KOBE 651-21, JAPAN

ABSTRACT. Hepatocytes were isolated from rats fed a diet with or without 0.25% clofibrate, and NAD^+ synthesis by the hepatocytes was determined using either [*carboxyl*-^{14}C]nicotinic acid or [5-^{3}H]tryptophan. NAD^+ and total pyridine nucleotides synthesized from [^{14}C]nicotinic acid by the clofibrate-treated cells were not significantly different from those synthesized by the control cells when expressed on the basis of nanomoles per hour per milligram of DNA. On the contrary, NAD^+ synthesized from [^{3}H]tryptophan was significantly higher in the clofibrate-treated cells (158% of the control cells) on the basis of nanomoles per hour per milligram of DNA. Clofibrate was inhibitory to tryptophan metabolism as a whole, affecting the glutarate pathway more (decreased to 37% of control) than the kynureninase flux (decreased to 64% of control). As a result, the quinolinate–NAD flux, estimated as the difference in the amounts of tryptophan metabolized by the two metabolic pathways, increased in the clofibrate-treated hepatocytes. The increase in quinolinate during the incubation was 8 times more in the clofibrate-treated cells than in the control cells, which confirmed alteration in the metabolic fluxes of tryptophan in the clofibrate-treated cells. Hepatic quinolinate phosphoribosyltransferase (EC 2.4.2.19) activity increased with dietary clofibrate and returned to the control level 1 week after removing clofibrate from the diet. Nicotinate phosphoribosyltransferase (EC 2.4.2.11) and NAD^+ glycohydrolase (EC 3.2.2.5) activities remained unchanged with dietary clofibrate. BIOCHEM PHARMACOL 52;2:247–252, 1996.

KEY WORDS. clofibrate-feeding; isolated rat hepatocytes; NAD^+ synthesis from nicotinic acid; NAD^+ synthesis from tryptophan; metabolic fluxes of tryptophan

In a previous report [1], we described an increase in the hepatic NAD^+ level of rats fed a diet containing clofibrate. Clofibrate (see structure below) is a hypolipidemic drug and is also known as a peroxisome proliferator [2]. NAD^+ is a cofactor necessary for peroxisomal fatty acid oxidation and an NAD^+ increase in the liver is beneficial to the animal. The main objective of the present study was to determine whether increased biosynthesis or decreased degradation could explain the increase of NAD^+ in the liver of clofibrate-treated rats. We estimated the synthesis of NAD^+ from nicotinic acid and from tryptophan by measuring the incorporation of [*carboxyl*-^{14}C]nicotinic acid and [5-^{3}H]tryptophan into NAD^+ in hepatocytes isolated from rats fed a clofibrate-containing diet. The metabolic flux of tryptophan by the hepatocytes was also estimated according to the method of Smith *et al.* [3] using L-[side chain-3-^{14}C]tryptophan and L-[5-^{3}H]tryptophan. Activities of rate-limiting enzymes in NAD^+ biosynthesis, such as QPRT† (EC 2.4.2.19) and NPRT (EC 2.4.2.11), and activity of NAD^+ase (EC 3.2.2.5), which is responsible for NAD^+ degradation, were determined as well.

MATERIALS AND METHODS

Materials

Clofibrate [2-(4-chlorophenoxy)-2-methylpropanoic acid ethyl ester] was obtained from Wako Pure Chemical Industries, Ltd., Osaka, Japan. L[5-^{3}H]Tryptophan (1.22 MBq/nmol) and L[side chain-3-^{14}C]tryptophan (2.04 KBq/nmol) were obtained from American Radiolabeled Chemicals Inc., St. Louis, MO, U.S.A. [*carboxyl*-^{14}C]Nicotinic acid (2.03 KBq/nmol) was purchased from the Sigma Chemical Co., St. Louis, MO, U.S.A. Each radiolabeled tryptophan was purified further by HPLC before use. Dowex 50W × 8 (200–400 mesh) and Dowex 1 × 2 (200–400 mesh) ion

* Corresponding author: Dr. Chisae Umezawa, School of Pharmacy, Kobe Gakuin University, Nishi-ku, Kobe 651-21, Japan. Tel. 78-974-1551, Ext. 2454; FAX 78-974-5689; E-mail: umezawa@kobegakuin.ac.jp
Received 2 October 1995; accepted 29 January 1996.

† *Abbreviations*: QPRT, quinolinate phosphoribosyltransferase; NPRT, nicotinate phosphoribosyltransferase; and NAD^+ase, NAD^+ glycohydrolase.

exchange resins were from Muromachi Kagaku, Tokyo, Japan. Charcoal (Norit A) and collagenase were from Nakalai Tesque, Kyoto, Japan, and Wako Pure Chemical Industries, respectively. NAD^+ase (from *Neurospora crassa*) was obtained from Sigma. Alcohol dehydrogenase (EC 1.1.1.1) for the NAD^+ assay was obtained from Boehringer Mannheim, Tokyo, Japan.

Animals and Diets

Sprague–Dawley male rats (specific pathogen-free; 200–220 g) were obtained from Japan SLC, Inc., Shizuoka, Japan. The 0.25% (w/w) clofibrate diet was prepared as described previously [1]. Diet and water were given freely. In a separate experiment, similar in design to that used in Ref. 1, we determined that clofibrate affected neither diet intake (control: 255 ± 9.7; clofibrate; 259 ± 16.7 g/2 weeks/rat) nor body weight gain (control: 49.9 ± 9.4; clofibrate; 50.0 ± 8.2 g/2 weeks/rat).

Preparation of Hepatocytes and Conditions of Incubation

Hepatocytes were prepared from rats fed either a control diet or a 0.25% clofibrate-containing diet for 2 weeks, using collagenase as described by Seglen [4]. The cells were suspended in a Krebs–Ringer bicarbonate buffer supplemented with 5 mM glucose and 0.5 mM glutamine in an Erlenmeyer flask, so that each milliliter of a cell suspension contained about 30 mg wet weight of cells. Nicotinic acid or tryptophan was added to give a final concentration of 26 μM nicotinic acid (containing 5.09 KBq [^{14}C]nicotinic acid/mL cell suspension) or 0.1 mM tryptophan (containing 35.7 KBq [^{3}H]tryptophan/mL cell suspension) with reference to the blood levels of a normal rat. Incubations were made by shaking the flasks in a water bath at 37° for 60 min at 80 oscillations/min in an atmosphere of 95% O_2:5% CO_2 (v/v).

NAD^+Synthesis from [carboxyl-^{14}C]Nicotinic Acid or [5-^{3}H]Tryptophan

NAD^+ was extracted from the incubation mixture with 0.5 M $HClO_4$. The supernatant after centrifugation was adjusted to pH 5–6 with 3 M KOH. After removing $KClO_4$ sediment by centrifugation, the supernatant fluid was enriched with non-labeled NAD^+ and subjected to a Dowex 50W × 8 column (H^+ form: 7 × 60 mm). The flow-through fraction contained total pyridine nucleotides and was subjected to a Dowex 1 × 2 column ($HCOO^-$ form: 7 × 40 mm). After washing the column with water, NAD^+ was eluted from the column with 0.02 N HCOOH. Recovery of NAD^+ was satisfactory when checked by determining $A_{260\,nm}$. When [5-^{3}H]tryptophan was used as an NAD^+ precursor, the eluate from a Dowex 1 × 2 column was adjusted to neutral pH and treated with NAD^+ase, and the resultant [^{3}H]nicotinamide was separated from other ^{3}H-contaminants by treatment with a Dowex 50W × 8 column (H^+ form: 5 × 30 mm). Conversion of NAD^+ to nicotinamide by NAD^+ase was complete when tested by alcohol dehydrogenase. Nicotinamide was eluted with 6 N HCOOH containing 0.5 N $HCOONH_4$. Radioactivity was determined with a Packard Tri-Carb liquid scintillation spectrometer.

Determination of Metabolic Fluxes of Tryptophan

The metabolic fluxes of tryptophan through specific enzymes were estimated by determining the rates of radioisotope release by the liver cells incubated with different radioisotopes of L-tryptophan after Smith *et al.* [3], except for using L[5-^{3}H]tryptophan and L-[side chain-3-^{14}C]tryptophan. Although tryptophan is known to be metabolized by various pathways, hepatocytes metabolize tryptophan only by the kynureninase pathway, especially at the low, physiological concentration used in the present study [3]. Smith *et al.* [3] reported that the rates of release of ^{3}H from L[5-^{3}H]tryptophan were similar to the rates of conversion of L-[benzene ring-U-^{14}C]tryptophan into $^{14}CO_2$ and ^{14}C-labeled non-aromatic products. This indicates that there was no detectable hydroxylation at position 5 of the benzene ring at the physiological concentration of tryptophan. Therefore, the radioactivity of non-aromatic metabolites (non-charcoal adsorptive) from [5-^{3}H]tryptophan can be assessed due to metabolic intermediates of tryptophan in the glutarate pathway.

The metabolic fates of tryptophan, shown in Fig. 1, were estimated by assaying charcoal non-adsorbed radioactivity and $^{14}CO_2$ trapped with 25% phenylethylamine. Tryptophan and aromatic metabolites (such as kynurenine, 3-hydroxykynurenine, 3-hydorxyanthranilate, kynurenate, xanthurenate, quinolinate, niacin, and NAD^+), were adsorbed onto charcoal (non-pretreated), with recovery of more than 97%. On the other hand, 94–98% of non-aromatic metabolites (such as alanine or formate) were recovered in the supernatants. Tryptophan metabolized via the kynureninase flux (alanine liberation) was estimated as the sum of nanomoles of tryptophan metabolized to charcoal non-adsorbed compounds (aliphatic compounds) plus nanomoles of tryptophan metabolized to $^{14}CO_2$ using L[side chain-3-^{14}C]tryptophan. Tryptophan metabolized via the glutarate pathway was estimated as nanomoles of tryptophan metabolized to charcoal non-adsorbed compounds using L[5-^{3}H]tryptophan. Metabolized tryptophan was calculated by subtracting residual tryptophan in the acid extract of the incubation mixture from added tryptophan, which was assayed according to Denckla and Dewey [5]. DNA content of the liver cells was determined as described by Burton [6].

Assay Methods for QPRT, NPRT, and NAD^+ase

Two groups of rats, each containing three rats, were fed either a control diet or a 0.25% clofibrate-diet for 2 weeks.

A third group of three rats was fed a 0.25% clofibrate-diet for 2 weeks and then switched to a control diet for 1 week. All animals were decapitated, and the liver was excised immediately. The liver homogenate for enzyme assays was prepared and treated with charcoal according to Ghafoorunissa and Narasinga Rao [7]. QPRT, NPRT, and NAD+ase activities were assayed according to Nishizuka and Nakamura [8], Honjo [9], and Kubota *et al.* [10], respectively. The NAD^+ content of the hepatocytes was determined according to Klingenberg [11]. Quinolinate was determined microbiologically, using *Lactobacillus plantarum* (ATCC 8014) as a test organism [12], as increased niacin after decarboxylation [13].

RESULTS

The amounts of NAD^+ and total pyridine nucleotides (nicotinic acid mononucleotide, nicotinic acid adenine dinucleotide, NAD^+, and $NADP^+$) synthesized from [^{14}C]-nicotinic acid in hepatocytes isolated from rats fed control and clofibrate-containing diets are shown in Table 1. As clofibrate is known to increase the weight of the liver resulting from both hyperplasia and hypertrophy [14], NAD^+ and total pyridine nucleotides synthesized were expressed on the basis of nanomoles per hour per milligram of DNA. No significant differences were observed in either NAD^+ or total pyridine nucleotides synthesized in cells from either clofibrate-treated or control rats.

On the other hand, the amount of NAD^+ synthesized from [^{3}H]tryptophan was increased significantly in the liver cells from clofibrate-treated rats compared with those in cells from control rats (Table 2). The total NAD^+ content determined after a 60-min incubation was significantly more in cells from clofibrate-treated rats. Specific radioactivity of NAD^+ was comparable in both control and clofibrate-treated cells. Considering that the size of the NAD^+ pool in the clofibrate-treated cells was almost twice as large as that in the control cells, the similar specific radioactivity of NAD^+ observed in the two groups of cells indicates that acceleration of NAD^+ biosynthesis occurred in the clofibrate-treated cells.

An increase in the NAD^+ synthesis from tryptophan observed in the clofibrate-treated cells was further confirmed by investigating the metabolic fluxes of tryptophan. In the conditions used, the amount of NAD^+ synthesized per milliliter of cell suspension increases linearly during the first 60 min of incubation [15]. As shown in Table 3, the total amounts of tryptophan metabolized tended to be lower in the clofibrate-treated cells, but were not significantly different in the two groups.

Metabolic fluxes of tryptophan were quite different in the two groups of cells. It was found that the amounts of tryptophan metabolized via the kynureninase flux and the glutarate pathway in the clofibrate-treated cells were decreased to 64 and 37% of the levels of the control cells, respectively, when compared on the basis of gram wet weight cells. The amount of tryptophan metabolized through the quinolinate–NAD^+ flux can be estimated as a difference of amounts of tryptophan metabolized via the kynureninase flux and that via the glutarate pathway (Fig. 1). The quinolinate–NAD^+ flux in the clofibrate-treated cells thus obtained was about 20 times as large as that of the control cells. When the quinolinate–NAD^+ flux was estimated per milligram DNA instead of per gram wet weight cells, the difference between the two groups was magnified even further because of hypertrophy caused by clofibrate (see structure in Fig. 2).

TABLE 1. Biosynthesis of NAD^+ from nicotinic acid in rat hepatocytes

Diet	NAD^+ synthesized (nmol/hr/mg DNA)	Total pyridine nucleotides synthesized (nmol/hr/mg DNA)
Control	43.3 ± 9.8	54.9 ± 11.1
Clofibrate	34.8 ± 9.1	46.6 ± 7.7

Hepatocytes were prepared from rats fed either a control diet or a 0.25% clofibrate-containing diet for 2 weeks. NAD^+ and total pyridine nucleotides synthesized were determined as described under Materials and Methods. Control and clofibrate values are means ± SD from five and four animals, respectively.

TABLE 2. Biosynthesis of NAD^+ from tryptophan in rat hepatocytes

Diet	NAD^+ synthesized (nmol/hr/mg DNA)	Specific radioactivity of NAD^+ (Bq/nmol)	NAD^+ content (nmol/mg DNA)
Control	28.1 ± 4.2	5.5 ± 1.0	456 ± 47
Clofibrate	44.4 ± 5.5*	4.8 ± 1.4	849 ± 250†

Hepatocytes were prepared from rats fed either a control or a 0.25% clofibrate-containing diet for 2 weeks. The amount of NAD^+ synthesized during a 60-min incubation and the NAD^+ contents of the hepatocytes were determined as described under Materials and Methods. Control and clofibrate values are means ± SD from four and three animals, respectively.

*,† Significantly different from control values as determined by Student's *t*-test: (*) $P < 0.01$, and (†) $P < 0.05$.

TABLE 3. Effects of dietary clofibrate on metabolic fluxes and metabolites of tryptophan in rat hepatocytes

	Control	Clofibrate
Metabolized Trp	1.64 ± 0.20	1.13 ± 0.35
Kynureninase flux	1.18 ± 0.09	0.75 ± 0.12*
Glutarate pathway	1.17 ± 0.06	0.43 ± 0.03†
QA–NAD^+ flux	0.02 ± 0.01	0.32 ± 0.12*
QA content	0.30 ± 0.01	0.93 ± 0.39
QA increased (during 60-min incubation)	0.10 ± 0.06	0.80 ± 0.24*

Hepatocytes were prepared from rats fed either a control diet or a 0.25% clofibrate-containing diet for 2 weeks. Metabolic fluxes of tryptophan (μmol/g wet weight cells/hr) and amounts of quinolinate (μmol/g wet weight cells) were determined as described under Materials and Methods. Control values are expressed as means ± range of two animals; clofibrate values are given as means ± SD of three animals.

*,† Significantly different from control values as determined by Student's *t*-test: (*) $P < 0.05$ and (†) $P < 0.001$.

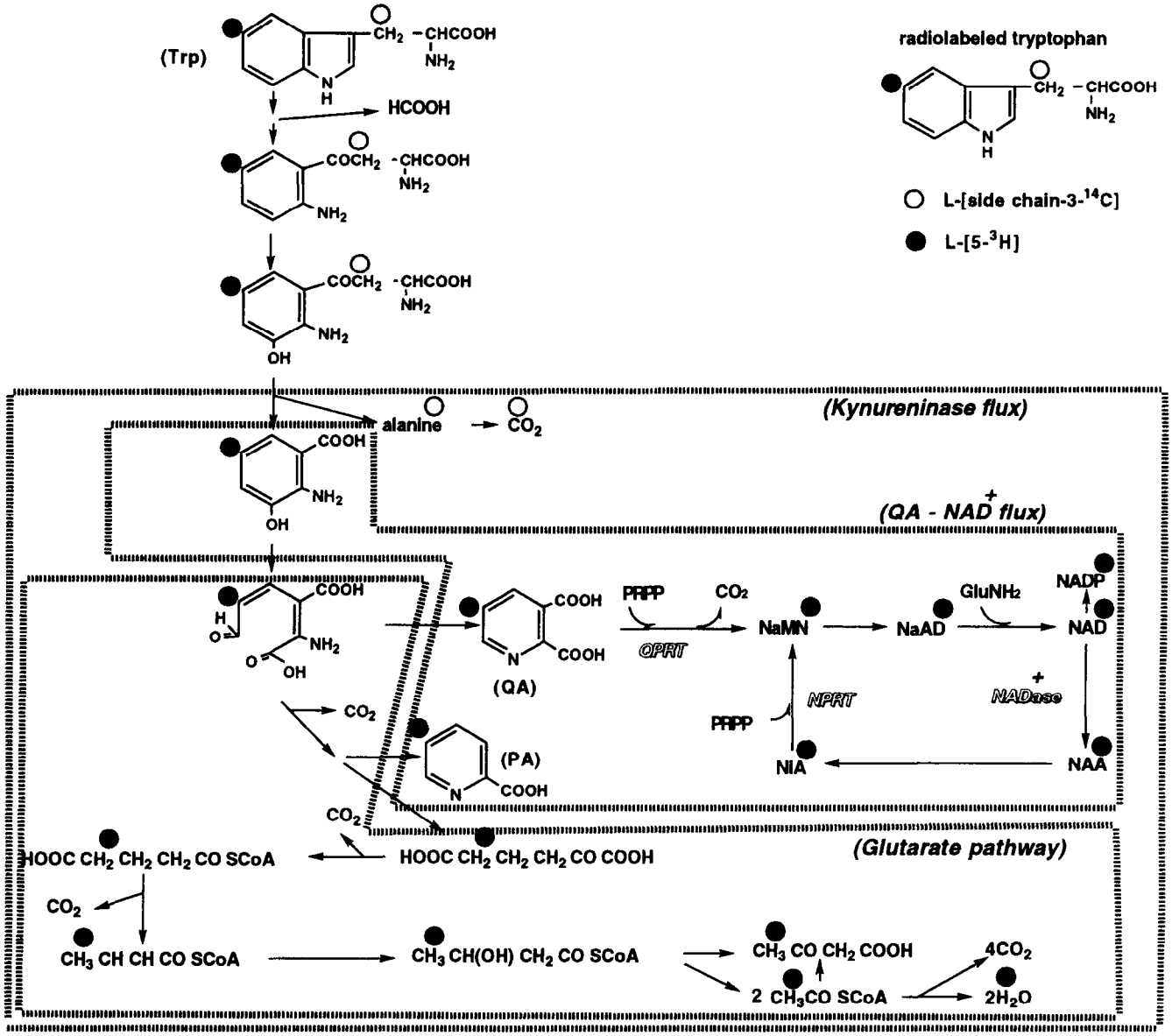

FIG. 1. **Metabolic fate of radioisotope from specifically radiolabeled L-tryptophan. An outline of L-tryptophan expected in the hepatocytes is shown. Abbreviations: Trp, tryptophan; QA, quinolinic acid (quinolinate); PA, picolinic acid; NaMN, nicotinic acid mononucleotide; NaAD, nicotinic acid adenine dinucleotide; NiA, nicotinic acid (niacin); NAA, nicotinamide; QPRT, quinolinate phosphoribosyltransferase; NPRT, nicotinate phosphoribosyltransferase; and NAD+ase, NAD+ glycohydrolase.**

To find a way to estimate the quinolinate–NAD^+ flux mentioned above, the amount of quinolinate increased during the 60-min incubation was determined. As shown in Table 3, the increase in quinolinate in clofibrate-treated cells during the 60-min incubation was 8-fold higher than that in the control cells. This figure would be even larger if calculated on the basis of milligrams of DNA.

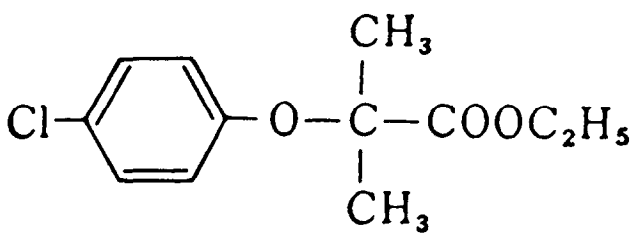

FIG. 2. **Structure of clofibrate.**

As shown in Table 4, no significant difference in NPRT activity was observed among the different feeding groups. On the other hand, the activity of QPRT was elevated significantly in the clofibrate group, but returned to the level of the control group after switching to the control diet for 1 week. The changes in the enzyme activity due to clofibrate in the diet corresponded well to the changes in hepatic NAD^+, i.e. QPRT activity was elevated in the clofibrate-fed rat liver, in which NAD^+ was increased, whereas the enzyme activity returned to the control level in the

TABLE 4. Effects of different feeding schedules on hepatic NAD+ level and enzyme activities

Group	1	2	3
Diet	Control	Clofibrate	Clofibrate → control
Liver NAD+ (nmol/mg DNA)	621 ± 126	1393 ± 120*	532 ± 69
QPRT (units/mg DNA)	5.63 ± 1.21	13.89 ± 1.77*	4.59 ± 0.67
NPRT (units/mg DNA)	6.45 ± 1.29	6.31 ± 0.56	5.05 ± 0.81
NAD+ase (units/mg DNA)	34.8 ± 4.2	33.2 ± 1.6	23.1 ± 1.8†

Rats were fed a control diet (group 1) or a 0.25% clofibrate-containing diet (group 2) for 2 weeks. Rats in group 3 were fed a 0.25% clofibrate-containing diet for 2 weeks and then a control diet for 1 week. Hepatic NAD+ and enzyme activities were determined as described under Materials and Methods. Values for each group are means ± SD from three animals.

*,† Significantly different from control value as determined by Student's *t*-test: (*) $P < 0.001$, and (†) $P < 0.005$.

liver of rats fed the control diet for 1 week after 2 weeks of the clofibrate-diet, during which NAD+ had returned to the control level. NAD+ase activity was the same in both groups with and without clofibrate but decreased in the group fed a clofibrate diet for 2 weeks and then a control diet for 1 week.

DISCUSSION

The elevated NAD+ level observed in the liver of rats fed the clofibrate diet is due to either acceleration of NAD+ synthesis or retardation of NAD+ degradation. In rat hepatocytes, NAD+ can be synthesized from both nicotinic acid and tryptophan [15]. In the present study, we found that NAD+ synthesis from [^{14}C]nicotinic acid was not significantly different with or without feeding a clofibrate-containing diet, while NAD+ synthesis from [5-^{3}H]tryptophan increased in the hepatocytes isolated from the clofibrate-treated rats. NAD+ synthesis from nicotinic acid is a simple pathway, but that from tryptophan is a complex and narrow side path branching from the main degradation pathway of tryptophan.

We found that the method of quantifying amounts of tryptophan metabolized via various pathways, which was developed by Smith *et al.*, is useful in both rat hepatocytes [16] and yeasts [17]. We, therefore, investigated (i) changes in tryptophan metabolism in hepatocytes caused by feeding a clofibrate-containing diet by applying the method by Smith *et al.* and (ii) how the changes in tryptophan metabolism found in the clofibrate-treated hepatocytes affect NAD+ synthesis from tryptophan.

The results in Table 3 suggest that clofibrate interferes with tryptophan metabolism at several different steps, possibly with tryptophan intake, kynureninase and aminocarboxymuconate-semialdehyde decarboxylase, though we do not have any definite evidence at this time. Tryptophan metabolized via the kynureninase flux in the clofibrate-treated hepatocytes decreased to 64% of that of the control cells, but tryptophan metabolism through the glutarate pathway was inhibited even more so that the amount of tryptophan metabolized via the quinolinate–NAD+ pathway increased in the clofibrate-treated cells. The amount of tryptophan metabolized via the quinolinate–NAD+ pathway was obtained by subtracting the amount of tryptophan metabolized via the glutarate pathway from that via the kynureninase flux. The amount of tryptophan metabolized through quinolinic acid to NAD+ was estimated by measuring the amount of quinolinic acid increased during the incubation period. Both quinolinic acid content and the amount of quinolinic acid increased during the incubation were higher in the clofibrate-treated cells, which verified our assumption of an increase via the quinolinate–NAD+ pathway in the clofibrate-treated cells.

The rate-limiting step in the synthesis of NAD+ from nicotinic acid is catalyzed by NPRT [18]. Judging from the V_{max} value [18], QPRT is also a candidate for rate-limiting enzymes in the tryptophan–NAD+ pathway. As shown in Table 4, the two enzyme activities behaved quite differently according to the dietary clofibrate. An elevation of QPRT activity in the liver of rats fed the clofibrate diet favors NAD+ synthesis from tryptophan. No difference in NAD+ase activity was observed with or without dietary clofibrate, suggesting that the enzyme is not responsible for the hepatic NAD+ increase after feeding the clofibrate-containing diet. Clofibrate added directly to the assay medium for metabolic fluxes of tryptophan or QPRT activity was without effect.

The present results strongly suggest that the elevated NAD+ in the liver of rats fed a clofibrate diet is due to an increase in the NAD+ biosynthesis from tryptophan. In addition, our results provide evidence of the importance of the glutarate pathway in controlling NAD+ synthesis from tryptophan. Further studies on the mechanism of clofibrate to increase NAD+ synthesis from tryptophan are under way.

References

1. Loo Y, Shin M, Yamashita Y, Ishigami M, Sasaki M, Sano K and Umezawa C, Effect of feeding the clofibrate-containing diet on the hepatic NAD+ level in rats. *J Nutr Sci Vitaminol (Tokyo)* **41:** 341–347, 1995.
2. Bremer J, Osmundsen H, Christiansen RZ and Barrebaek B, Clofibrate. *Methods Enzymol* **72:** 506–519, 1981.
3. Smith SA, Carr FP and Pogson CI, The metabolism of L-tryptophan by isolated rat liver cells. Quantification of the relative importance of, and the effect of nutritional status on, the individual pathways of tryptophan metabolism. *Biochem J* **192:** 673–686, 1980.
4. Seglen PO, Preparation of isolated rat liver cells. *Methods Cell Biol* **13:** 29–83, 1976.
5. Denckla WD and Dewey HK, The determination of tryptophan in plasma, liver and urine. *J Lab Clin Med* **69:** 160–169, 1967.
6. Burton K, Determination of DNA concentration with diphenylamine. *Methods Enzymol* **12B:** 163–166, 1968.
7. Ghafoorunissa and Narasinga Rao BS, Effect of leucine on enzymes of the tryptophan-niacin metabolic pathway in rat liver and kidney. *Biochem J* **134:** 425–430, 1973.
8. Nishizuka Y and Nakamura S, Quinolinate phosphoribosyl-

transferase (beef liver). *Methods Enzymol* **17A:** 491–500, 1970.
9. Honjo T, Nicotinate phosphoribosyltransferase from baker's yeast. *Methods Enzymol* **18B:** 123–127, 1971.
10. Kubota H, Nomura K, Yamada O, Shin M, Sano K and Umezawa C, NAD glycohydrolase activity in the liver of rats fed on excess leucine diet and low or high protein diet. *J Nutr Sci Vitaminol (Tokyo)* **28:** 57–66, 1982.
11. Klingenberg M, 3.3 Nicotinamide-adenine dinucleotides and dinucleotide phosphates (NAD, NADP, NADH, NADPH). 3.3.1 End-point UV-methods. In: *Methods of Enzymatic Analysis* (Ed. Bergmeyer HU), 3rd Edn, Vol. VII, pp. 251–257. VCH Verlagsgesellschaft, Weinheim, Germany, 1985.
12. Snell EE and Wright LD, A microbiological method for the determination of nicotinic acid. *J Biol Chem* **139:** 675–686, 1941.
13. Henderson LM, Quinolinic acid excretion by the rat receiving tryptophan. *J Biol Chem* **178:** 1005–1006, 1949.
14. Lock EA, Mitchell AM and Elcombe CR, Biochemical mechanisms of induction of hepatic peroxisome proliferation. *Annu Rev Pharmacol Toxicol* **29:** 145–163, 1989.
15. Yamada O, Shin M, Sano K and Umezawa C, Effect of leucine and α-ketoisocaproic acid on NAD biosynthesis from tryptophan or nicotinic acid in the isolated rat liver cells. *Int J Vitam Nutr Res* **53:** 184–191, 1983.
16. Kiyohara Y, Shirasawa K, Shin M, Sano K and Umezawa C, Tryptophan metabolism by the isolated rat liver cells—Effects of leucine and its metabolites. *Int J Vitam Nutr Res* **59:** 88–92, 1989.
17. Shin M, Shingu T, Sano K and Umezawa C, Metabolic fates of L-tryptophan in *Saccharomyces uvarum* (*Saccharomyces carlsbergensis*). *Chem Pharm Bull (Tokyo)* **39:** 1792–1795, 1991.
18. Shibata K, Hayakawa T and Iwai K, Comparison of the enzyme activities in the tryptophan–NAD pathway between the Wistar and Sprague Dawley strains of rats. *Agric Biol Chem* **50:** 1643–1644, 1986.