Water Maze Performance in Young Male Long-Evans Rats Is Inversely Affected by Dietary Intakes of Niacin and May Be Linked to Levels of the NAD⁺ Metabolite cADPR¹

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Abstract

Niacin is converted in tissues to NAD⁺, which is required for synthesis of the intracellular calcium signaling molecule cyclic ADP-ribose (cADPR). cADPR is involved in many aspects of cognitive function, including long-term depression, in the hippocampus, a brain region that regulates spatial learning ability. The objective of this study was to determine whether niacin deficiency and pharmacological nicotinamide supplementation have an effect on spatial learning ability in young male Long-Evans rats as assessed by the Morris Water Maze, and whether brain NAD⁺ and cADPR are modified by dietary niacin intake. We investigated 3 models of niacin deficiency: niacin deficient (ND) vs. pair fed (PF), ND vs. partially feed restricted (PFR), and ND vs. niacin recovered (REC). ND rats showed an improvement in spatial learning ability relative to PF, PFR, and REC rats. ND rats also showed a decrease in both NAD⁺ and cADPR relative to PF and REC rats. We also investigated 1 model of pharmacological supplementation, niacin-supplemented vs. control. The niacin-supplemented group showed a small but significant spatial learning impairment relative to controls, and an increase in brain cADPR and NAD⁺. Changes in neural function related to the NAD⁺ associated calcium signaling molecule, cADPR, may be the link between diet and behavior. J. Nutr. 137: 1050–1057, 2007.

Introduction

Niacin exists in several forms, including nicotinamide (pyridine-3-carboxyamide) and nicotinic acid (pyridine-3-carboxylic acid). Nicotinamide is found mainly in plant products, whereas animal products contain mainly nicotinamide, or nucleotides that generate nicotinamide during digestion. Niacin is converted in tissues to NAD and NADP, which are involved in a multitude of cellular processes. The essential amino acid tryptophan can also be used as a substrate for the synthesis of NAD. NAD⁺ is involved in energy metabolism (redox reactions), protein modification by mono- and poly-(ADP-ribose) polymerases, and the synthesis of cyclic ADP-ribose (cADPR)¹ (1). cADPR is a calcium signaling molecule that induces a release of Ca²⁺ from intracellular stores (2) through binding to ryanodine receptors type II and III (3). In the brain, cADPR has been linked to neuronal processes such as long-term depression (LTD) (4,5) and neurotransmitter release (6–8). Additionally, through ryanodine receptors, cADPR may be implicated in the regulation of the circadian clock (9) and in long-term potentiation (10). cADPR has furthermore been involved in bidirectional coupling between ryanodine receptors and voltage-activated calcium channels in mammalian neuronal tumor cells (11).

Niacin was identified as a vitamin following years of devastation by the disease pellagra. Pellagra, the disease of niacin deficiency, is characterized by the 4 Ds of dermatitis, diarrhea, dementia, and death (1). The dementia of pellagra is similar to schizophrenia, in which patients show signs of depression and apprehension that progress to paranoia, delirium, hallucinations, and violent behavior as the disease worsens. Strikingly, the majority of psychological functioning is recovered within days when insane patients with pellagra are treated with niacin, which suggests that the dementia is caused by alterations in neural signaling pathways, rather than structural pathological changes (1). Although the evidence from pellagra clearly indicates that niacin is involved in neural functioning, minimal investigation has considered the effects of either niacin deficiency or pharmacological niacin supplementation on cognitive abilities. Such investigations are warranted, as both clinical (12,13) and subclinical (14,15) niacin deficiencies continue to occur, and pharmacological treatment with nicotinamide has been investigated as a preventative treatment for type 1 diabetes in children (16–18). Additionally, although studies have shown that dietary

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² Abbreviations used: cADPR, cyclic ADP-ribose; CON, control; LTD, long-term depression; MWM, Morris Water Maze; ND, niacin deficient; PCA, perchloric acid; PF, pair fed; PFR, partially feed restricted; REC, recovered; SUPP, supplemented.
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nacin can modify NAD$^+$ in various tissues (19–21), effects on cADPR are unknown. It was therefore the objective of our study to determine whether nacin deficiency and pharmacological nicotinamide supplementation have an effect on spatial learning ability in young male Long-Evans rats as assessed by the Morris Water Maze (MWM), and whether brain CADPR, like brain NAD$^+$, is modified by dietary nacin intake.

**Materials and Methods**

**Animals**

Male weanling Long-Evans rats (initial weight: 35–40 g) (Charles River Canada) were housed singly in wire-bottomed cages with unrestricted access to water. Rats were fed between 1000 and 1200 each day, weighed biweekly, and maintained on a 12:12 light:dark cycle (light from 0600–1800) at 22 ± 2°C. The study was approved by the Animal Care Committee at the University of Guelph and adhered to the standards set forth by the Canadian Council on Animal Care. During the course of each experiment, any animal that demonstrated a >10% weight loss from the peak weight on 2 successive weighings was considered moribund and was euthanized.

**Dietary manipulations**

Rats were fed a nutritionally complete AIN-93 (22) diet with modifications as followed below.

**Expt. 1: Niacin deficiency vs. pair feeding.** The nacin-deficient (ND) group was fed a diet containing 0 mg nicotinic acid/kg, whereas the pair-fed (PF) group was fed a control diet containing 30 mg nicotinic acid/kg diet (Table 1). There were 8 rats in each group, and each ND rat was weight-matched to a PF counterpart. The amount of food consumed by each ND rat was determined daily, and the same amount of food was given weight-matched to a PF counterpart. The pair-feeding strategy was used to maintain equivalent food consumption between the 2 groups. In wk 5 of the experiment, 3 rats in the ND group became moribund and were euthanized along with their PF partners, leaving $n=5$. In the 4th week of the experiment, 6 ND rats became moribund and were euthanized along with their corresponding REC partners, leaving $n=9$ in both groups.

**TABLE 1** Composition of experimental diets

<table>
<thead>
<tr>
<th></th>
<th>Niacin deficient</th>
<th>Control$^1$</th>
<th>Niacin supplemented</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>g/kg diet</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cerelose</td>
<td>720</td>
<td>719.997</td>
<td>716</td>
</tr>
<tr>
<td>Casein</td>
<td>60</td>
<td>60</td>
<td>60</td>
</tr>
<tr>
<td>Gelatin</td>
<td>70</td>
<td>70</td>
<td>70</td>
</tr>
<tr>
<td>Cellulose</td>
<td>50</td>
<td>50</td>
<td>50</td>
</tr>
<tr>
<td>AIN-93 Mineral mix$^2$</td>
<td>35</td>
<td>35</td>
<td>35</td>
</tr>
<tr>
<td>Vitamin mix$^3$</td>
<td>10</td>
<td>10</td>
<td>10</td>
</tr>
<tr>
<td>Methionine</td>
<td>3</td>
<td>3</td>
<td>3</td>
</tr>
<tr>
<td>Choline bitartrate</td>
<td>2</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>Nicotinic acid</td>
<td>0</td>
<td>0.003</td>
<td>0</td>
</tr>
<tr>
<td>Nicotinamide$^4$</td>
<td>0</td>
<td>0</td>
<td>4</td>
</tr>
<tr>
<td>Soya oil</td>
<td>50</td>
<td>50</td>
<td>50</td>
</tr>
</tbody>
</table>

1 The control diet was fed to the PF rats in Expt. 1, the PFR rats in Expt. 2, the REC rats in Expt. 3, and the CON rats in Expt. 4.
2 See Reeves et al. (47).
3 Vitamin mix composition: 97.543% sucrose, 0.0001% vitamin B-12, 2% vitamin E di-α-tocopherol acetate, 0.002% biotin, 0.16% calcium pantothenate, 0.02% folic acid, 0.005% vitamin K phyloquinone, 0.07% pyrdoxine HCl, 0.06% riboflavin, 0.06% thiamine HCl, 0.08% retinyl palmitate, 0.00025% cholecalciferol.
4 In Expt. 3, the nacin-deficient diet alternately contained 0 and 1 mg nicotinic acid/kg.

Morris Water Maze apparatus and procedures

The Morris Water Maze (MWM) apparatus was the same as previously described (23). In brief, the MWM consisted of a circular pool, 180 cm in diameter and 60 cm in height, with the interior painted white. The escape platform was made of clear plexiglass, 14 cm in diameter and 15 cm in height, and was located ~1 cm below the surface of the water. Visual cues were visible to the rats, including 4 geometric shapes that measured at least 20 cm in height and were positioned so that they were 15 cm above water level and remained distal to the rats at all times. The water was maintained at a temperature of 22 ± 2°C, and was made opaque by the addition of nontoxic white latex paint (Crayola). Movement of rats in the pool was analyzed by a digital tracking system (Columbus Instruments). The software used in analysis of behavior was Water Maze, version 4.20 (Columbus Instruments). The maximum trial time in each phase of the experiment was 90 s. If the time limit was exceeded, the animal was gently guided to the platform where it remained for 15 s. The starting location was varied in each trial, and the sequence of start locations was randomized across days. Following the completion of the trial, rats were allowed to warm and dry off under heat lamps in holding cages before being returned to their home cage. There were 3 trials/d with an intertrial interval of ~2 h. Due to the time required to complete 3 trials, rats were tested during both the 1st and 2nd part of their light cycle.

In Expt. 1 and 2, testing in the MWM began on d 21 of the experiment; in Expt. 3, testing in the MWM began on d 20 of the Niacin affects learning and brain nucleotides 1051
experiment; and in Expt. 4, testing in the MWM began on d 15 of the experiment. In each experiment, there was an acquisition phase that lasted 6 d. In Expt. 3, there was also a reversal training phase, in which the platform was moved to a new location, that lasted 4 d. The platform was moved to the opposite quadrant of the pool and was placed ~10 cm closer to the pool wall during reversal training. All other water maze procedures remained the same during this period. Reversal training in Expt. 3 began after 4 d of niacin refeeding, on d 30 of the experiment. During reversal training, the REC were fed a niacin-replete diet, whereas during acquisition they were fed a ND diet. Following completion of the last run of training in each experiment, a probe trial was run in which the platform was removed from the pool and the rats were allowed to swim freely for 30 s. The pool was divided into 4 quadrants of equal size, and the number of crossings of the target platform in the target quadrant were measured and compared against the number of crossings of the symmetrical platform locations in the other 3 quadrants of the pool.

For each trial, measures of cumulative error, measured in cm, were obtained. Cumulative error was the combined measure of the latency of swimming and how far the rat deviated from an ideal swim path, and it helped to eliminate the influence of swim speed on the results (24). The results of the 3 daily trials were averaged to give a mean value for each day of testing.

Schematic representations of the diet and water maze procedures in each of the 3 niacin deficiency experiments are shown in Figure 1.

**Tissue preparation**

For preparation of brain tissue isolated from rats in Expt. 1, rats were anesthetized with Isofluorane (Fisher Scientific) and the heads were then decapitated with a guillotine and the brain was rapidly dissected out of the skull while oxygenated blood is still circulating (25,26). Heads were collected and returned immediately into liquid nitrogen and then stored at −80°C. Later, the brain was dissected out of the skull while still frozen and homogenized in a solution of ice cold 1 mol/L perchloric acid (PCA) at a concentration of 0.1 g of tissue to 1 mL PCA, using an electric homogenizer (Pro250, 10 mm head). Samples were centrifuged twice for 10 min, with the 1st at 1000 × g and the 2nd at 14000 × g. The aqueous layer was recovered from both centrifugations. For preparation of brain tissue isolated from rats in Expt. 2 and 4, anesthetized rats were decapitated with a guillotine and the brain was rapidly dissected out of the skull and placed immediately into liquid nitrogen and stored at −80°C. This method of termination was after observing that NAD⁺ and cADPR levels did not differ significantly between rats that had undergone termination by submersion in liquid nitrogen and those that had been decapitated under anesthesia (unpublished observations). Frozen tissue was then treated in an identical manner to that described for Expt. 1.

**Measurement of cADPR**

The assay used to measure levels of cADPR was the cycling assay described in (27) with the modifications described in (28). Brain cADPR measurements were made on d 35 in Expt. 1, on d 33 in Expt. 3, and on d 21 in Expt. 4. There was no measurement of cADPR in Expt. 2.

**Measurement of NAD⁺**

Samples were diluted 5-fold in PCA from the initial concentration of 0.1 g of tissue to 1 mL of PCA. Upon thawing, all samples were neutralized to between pH 6.7 and 6.8 with 2 mol/L potassium hydroxide and analyzed for NAD⁺ content via the enzyme cycling of alcohol dehydrogenase (29). Brain NAD⁺ measurements were made on d 35 in Expt. 1, d 33 in Expt. 3, and d 21 in Expt. 4. There was no measurement of NAD⁺ in Expt. 2.

**Statistical analysis**

All statistical analyses were performed using SPSS, version 12.0 for Windows. Significance was determined at P ≤ 0.05. A trend was defined as a P-value between 0.05 and 0.1. The Kolmogorov-Smirnov and Shapiro-Wilk tests were used to evaluate normality. Because water maze data in each experiment showed an abnormal distribution on at least 1 experimental day, nonparametric tests were used to assess water maze performance. The Friedman test was performed to determine the within-subjects effect, and the Kruskal-Wallis test was performed to determine the between-subjects effect. The within-subjects factor was time (test day) and the between-subjects factor was diet. For probe trial analysis, 1-way ANOVA was used to compare the number of platform crossings at each of the 4 possible platform locations. Two-tailed independent t tests were used to compare mean swim speeds and brain nucleotides. Data were not transformed before analysis.

**Results**

**Expt. 1: Niacin deficiency vs. pair feeding.** ND rats performed better than PF controls in the water maze on d 2, 3, 5, and 6 (P < 0.05) and tended to do so on d 4 (P = 0.1). Both groups improved over time (P ≤ 0.001) (Fig. 2). ND rats swim faster than PF rats (P < 0.001) (Table 2). During the probe trial (90-s swim with platform removed) ND rats crossed platform 2 (the previous platform location) more times than the analogous positions in quadrants 1, 3, and 4 (P < 0.05), PF rats tended to cross platform 2 more times than platform 3 (P = 0.1). ND rats tended to cross the target platform more times than PF rats (P = 0.09) (data not shown). Brain NAD⁺ and cADPR (Table 3) concentrations were less in ND rats than in PF controls (P < 0.02).

**Expt. 2: Niacin deficiency vs. partial feed restriction.** When the pair-feeding regimen ended on d 17 in this model, the PF rats consumed more food than ND rats for the duration of the experiment (Fig. 3A). ND rats weighed less than PFR rats from...
d 17 to d 27 (Fig. 3B). ND rats performed better than PFR controls in the water maze on d 3 and 4 \((P < 0.05)\), and tended to do so on d 6 \((P = 0.08)\). Both groups improved over time \((P \leq 0.001)\) (Fig. 4). ND rats swam faster than PFR rats \((P < 0.001)\) (Table 2). In the probe trial, ND and PFR rats crossed platform 2 (the previous platform location) more times than platforms 1, 3 and 4 \((P < 0.05)\). ND and PFR rats did not differ in the number of crossings of the target platform (data not shown).

**Expt. 3: Niacin deficiency vs. niacin recovery.** In this model, all rats were fed the ND diet for the 1st 25 d, and they underwent acquisition training in the last 6 d of this period. Rats that eventually entered the ND group did not differ from REC groups in a retrospective analysis of performance during the acquisition phase of the experiment (data not shown). During reversal training in the water maze, ND rats performed better than REC controls on d 4 \((P = 0.01)\) and tended to do so on d 2 \((P = 0.09)\). Both groups improved over time \((P \leq 0.001)\) (Fig. 5). ND rats swam faster than REC rats \((P < 0.001)\) during reversal training but not in a retrospective analysis of swim speed during acquisition, when both groups were still deficient (Table 2). ND and REC rats crossed platform 3 (the target platform) more times than platform 1, 2, and 4 \((P < 0.05)\) and did not differ in the number of crossings of the target platform (data not shown). Brain NAD\(^+\) and cADPR (Table 3) concentrations in ND rats were less than in REC controls \((P \leq 0.001)\).

**Expt. 4: Niacin supplementation vs. control.** SUPP rats performed worse than CON controls in the water maze on d 3 \((P = 0.04)\). Both groups improved over time \((P \leq 0.001)\) (Fig. 6). SUPP rats swam faster than CON rats \((P = 0.001)\) (Table 2). SUPP rats crossed platform 2 (the target platform) more times than platforms 3 and 4 \((P < 0.01)\), and tended to cross platform

**TABLE 2** Swim speed of rats with differing niacin intakes\(^1\)

<table>
<thead>
<tr>
<th>Expt</th>
<th>(n)</th>
<th>(\text{cm/s})</th>
</tr>
</thead>
<tbody>
<tr>
<td>ND</td>
<td>8</td>
<td>28.8 ± 0.4</td>
</tr>
<tr>
<td>PF</td>
<td>8</td>
<td>25.6 ± 0.3*</td>
</tr>
<tr>
<td>ND</td>
<td>9</td>
<td>28.1 ± 0.4</td>
</tr>
<tr>
<td>PFR</td>
<td>8</td>
<td>22.6 ± 0.4*</td>
</tr>
<tr>
<td>ND</td>
<td>15</td>
<td>28.5 ± 0.4</td>
</tr>
<tr>
<td>REC</td>
<td>15</td>
<td>27.9 ± 0.4</td>
</tr>
<tr>
<td>ND</td>
<td>9</td>
<td>34.2 ± 0.6</td>
</tr>
<tr>
<td>REC</td>
<td>9</td>
<td>28.2 ± 0.4*</td>
</tr>
<tr>
<td>SUPP</td>
<td>18</td>
<td>27.7 ± 0.3*</td>
</tr>
<tr>
<td>CON</td>
<td>15</td>
<td>26.3 ± 0.3</td>
</tr>
</tbody>
</table>

\(^1\) Values are means ± SEM.

\(^*\) Different from ND or CON, \(P \leq 0.01\).

**Figure 3** Food intake (A) and body weight (B) of ND and PFR rats (Expt. 2). PFR rats were pair fed for the 1st 16 d of the experiment, and then were fed ad libitum from d 17 to d 27. Values are means ± SEM, \(n = 8\). *ND different from PFR, \(P \leq 0.05\).
PFR, in the water maze and 4 d of niacin refeeding. The results of the 3 daily trials were averaged to give a mean value for each day of testing. Values are means ± SEM, n = 9 ND; n = 8 PFR. *ND different from PFR, P ≤ 0.05.

2 more times than platform 1 (P = 0.07). Similarly, CON rats crossed platform 2 more times than platforms 1, 3, and 4 (P < 0.01). SUPP and CON rats did not differ in the number of crossings of the target platform (data not shown). Brain NAD$^+$ and cADPR (Table 3) concentrations in SUPP rats were higher than in CON controls (P < 0.02).

Discussion

This study was designed to investigate the effect of niacin deficiency and pharmacological nicotinamide supplementation on spatial learning ability and brain cADPR in weanling male Long-Evans rats. Using 3 different models of niacin deficiency, we demonstrated that niacin deficiency caused a significant improvement in spatial learning ability. In contrast, using a model of nicotinamide supplementation, we demonstrated that pharmacological doses of nicotinamide caused a significant impairment in spatial learning ability. Both cADPR and NAD$^+$ decreased in niacin deficiency and were elevated following pharmacological niacin supplementation. The results of this study demonstrate that spatial learning ability shows an inverse relation to deficient and pharmacological dietary intakes of niacin, whereas brain cADPR is directly modified by dietary niacin intake.

Niacin deficiency positively affected spatial learning ability in each of the 3 niacin deficiency experiments. In the 1st experiment, control rats were pair fed a diet containing 30 mg added nicotinic acid/kg diet throughout the duration of the experiment. This level is considered adequate to fully meet the needs of rats and is found in AIN93 formulations and most commercial rat chows. The pair-feeding model was used to control for differences in feed intake between niacin-deficient and control rats, insofar as a deficiency of niacin, like most micronutrients, causes anorexia (1). In the water maze, niacin-deficient rats showed superior spatial learning ability during acquisition and a trend for higher spatial accuracy in a probe test. However, we were concerned that rats voluntarily restricting their food intake due to anorexia may be different from rats being forcibly feed restricted and that the effect of hunger in the latter condition may have physiological consequences, at least in terms of cognitive function. To eliminate this effect, we used a modified pair-feeding model in Expt. 2 that we called partially restricted feeding. We pair fed the control group for the 1st 16 d of the experiment, and then fed them ad libitum for 4 d before and during the entire period of water maze testing. Our goal was to reduce hunger during the period of behavioral testing while minimizing the developmental differences that would result between niacin-deficient rats and rats fed ad libitum throughout the experiment. In the water maze, we observed that niacin-deficient rats again showed superior spatial learning ability, although the spatial accuracy of the 2 groups was comparable in a probe test. The food intake and body weight of the 2 groups diverged greatly once the control group was placed on ad libitum feeding. Whereas the reduced food intake in Expt. 1 may have been a confounding variable, due to the effect of hunger on spatial learning ability, the increased feed intake in Expt. 2 may have been a similar confounding variable. Although the control rats were no longer hungry, they were consuming significantly more macro- and micronutrients than the niacin-deficient group, which would have an effect on a vast number of metabolic and developmental processes. So, in the 3rd niacin deficiency experiment, we used a recovery model in which all rats were maintained on a niacin-deficient diet during the 1st phase of water maze testing, and then half were recovered from the deficiency through niacin refeeding during the 2nd phase of water maze testing. Nicotinamide was used because it is the preferred substrate for NAD$^+$ in most tissues, including the brain (30), and should therefore allow for a more rapid

![Figure 4](https://academic.oup.com/jn/article-abstract/137/4/1050/4664613) Cumulative error of ND and PFR rats in the water maze (Expt. 2). Rats were tested in 3 daily trials across 6 d with an intertrial interval of 2 h. The results of the 3 daily trials were averaged to give a mean value for each day of testing. Values are means ± SEM, n = 9 ND; n = 8 PFR. *ND different from PFR, P ≤ 0.05.

![Figure 5](https://academic.oup.com/jn/article-abstract/137/4/1050/4664613) Cumulative error of ND and REC rats during reversal training in the water maze (Expt. 3). Rats were tested in 3 daily trials across 4 d with an intertrial interval of 2 h. The reversal training followed an initial acquisition phase in the water maze and 4 d of niacin refeeding. The results of the 3 daily trials were averaged to give a mean value for each day of testing. Values are means ± SEM, n = 9. *ND different from REC, P ≤ 0.05.

![Figure 6](https://academic.oup.com/jn/article-abstract/137/4/1050/4664613) Cumulative error of SUPP and CON rats in the water maze (Expt. 4). Rats were tested in 3 daily trials across 6 d with an intertrial interval of 2 h. The results of the 3 daily trials were averaged to give a mean value for each day of testing. Values are means ± SEM, n = 18 SUPP; n = 15 CON. *SUPP different from CON, P ≤ 0.05.

Figure 4 Cumulative error of ND and PFR rats in the water maze (Expt. 2). Rats were tested in 3 daily trials across 6 d with an intertrial interval of 2 h. The results of the 3 daily trials were averaged to give a mean value for each day of testing. Values are means ± SEM, n = 9 ND; n = 8 PFR. *ND different from PFR, P ≤ 0.05.

Figure 5 Cumulative error of ND and REC rats during reversal training in the water maze (Expt. 3). Rats were tested in 3 daily trials across 4 d with an intertrial interval of 2 h. The reversal training followed an initial acquisition phase in the water maze and 4 d of niacin refeeding. The results of the 3 daily trials were averaged to give a mean value for each day of testing. Values are means ± SEM, n = 9. *ND different from REC, P ≤ 0.05.

Figure 6 Cumulative error of SUPP and CON rats in the water maze (Expt. 4). Rats were tested in 3 daily trials across 6 d with an intertrial interval of 2 h. The results of the 3 daily trials were averaged to give a mean value for each day of testing. Values are means ± SEM, n = 18 SUPP; n = 15 CON. *SUPP different from CON, P ≤ 0.05.
replenishment of niacin metabolites. The 4-d period of niacin refeeding prior to the 2nd phase of water maze testing was designed to mimic the period of nutritional rehabilitation typically required for resolution of the symptoms of pellagrous dementia (1). During the 1st phase of water maze testing, when all rats were niacin deficient, a retrospective analysis of performance revealed no significant differences between the 2 groups. However, during the 2nd phase of water maze testing, when the recovered rats were being refed niacin, niacin-deficient rats showed superior spatial learning ability, with comparable spatial accuracy in the probe test. It is important to note that during the 2nd phase of testing, rats were no longer naive and had already undergone extensive water maze training, so the sensitivity of the test to detect subtle differences in spatial learning ability would be reduced. If hunger associated with pair feeding is, in fact, a confounding variable, it might be expected to affect the results in this experiment in the same way as in Expt. 1, because REC rats were pair fed to ND rats during the period of behavioral testing. However, we found that niacin-deficient rats require a number of days to fully recover their appetite, so the recovery group was not likely faced with the same hunger issues of the earlier pair-fed rats. The swim speed of ND rats in all 3 experiments was increased relative to each control group.

In contrast to niacin deficiency, we found that niacin supplementation negatively affects spatial learning ability. In Expt. 4, the effect of pharmacological nicotinamide supplementation on spatial learning ability was investigated. The brain shows a preference for using nicotinamide in the synthesis of NAD⁺ over any other precursors, and there is an active mechanism for nicotinamide uptake into the brain, where it is distributed evenly (31). The level of nicotinamide used was comparable to the human consumption of 1–3 g of nicotinamide/d by children in the diabetes prevention trials (32). In the water maze, we observed that niacin-supplemented rats showed inferior spatial learning ability, whereas the spatial accuracy of the 2 groups was comparable in a probe test. To our knowledge, there is only 1 previously published report of the effect of oral nicotinamide supplementation on spatial learning ability (33). In that study, the dose of nicotinamide given was comparable to ours, but rats were tested immediately following nicotinamide administration rather than after an extended period of dietary manipulation. In adult rats, nicotinamide supplementation had no effect on spatial learning ability, but in aged rats, there was a significant learning impairment. The effect of nicotinamide supplementation in weanling rats was not investigated. The observation of impairments in spatial learning ability in young rats following nicotinamide supplementation may have implications for diabetes studies in which comparable doses of the vitamin are being investigated as a preventive agent for NIDDM in young children (16–18). Like in the ND rats, the swim speed of niacin-supplemented rats was increased relative to the control group. The finding of increased swim speed in both ND and SUPP rats suggests that swim speed cannot explain the effect of niacin intake on spatial learning ability.

Changes in levels of the NAD⁺ metabolite cADPR may be the link between spatial learning ability and diet. In this study, we demonstrated that brain NAD⁺ and cADPR were decreased significantly in niacin-deficient rats. In contrast, brain NAD⁺ and cADPR were increased significantly in niacin-supplemented rats. Like dietary niacin, brain NAD⁺ and cADPR therefore show an inverse relation to spatial learning ability, with improvements correlated with reduced nucleotides and impairments correlated with increased nucleotides. At this time, the association between brain cADPR and spatial learning ability is correlative, but there is considerable evidence that cADPR is involved in brain function (4–10), and levels of cADPR in the brain are higher than in many other organs (34). In the hippocampus, cADPR is required for a form of long-term depression (LTD) that involves nitric oxide as a retrograde messenger and activation of guanylyl cyclase (35,36). The site of action for cADPR is in the presynaptic neuron (37), and presynaptic modulation of LTD involves changes in neurotransmitter release, either through reductions in quantal size or frequency of transmission (38). Animals that show impaired LTD display improved spatial learning (39), whereas animals that show facilitated LTD display impaired spatial learning (40,41). Following this, we propose that, in niacin deficiency, there will be a decrease in systemic NAD⁺ that will subsequently decrease brain NAD⁺ and reduce the availability of the substrate for ADP-ribosyl cyclase enzymes to synthesize cADPR. Reduced cADPR will impair LTD, thereby facilitating LTP and improving spatial learning ability. Nicacin supplementation will have the opposite effect of increasing NAD⁺ and cADPR and facilitating LTD, causing an impairment of spatial learning ability (Fig. 7).

Although each model of niacin deficiency presents its own interpretational challenges, the consistency of finding improved spatial learning ability is striking. This, combined with the opposite observation following nicotinamide supplementation, supports the finding of an inverse relation between spatial learning ability and dietary niacin intake in very young rats. These findings by no means imply that niacin deficiency is beneficial. In addition to the evidence from pellagra patients, in which sufferers experience profound psychological disturbance, there is data that subclinical deficiencies may also be detrimental, at least in elderly populations (42,43). In contrast, nicotinamide supplementation exerts a protective action in the brain, capable of reducing damage following injury and minimizing inflammation (44). Supplementation with nicotinic acid has also been shown to benefit memory (45), an effect that may be due to increased cerebral blood flow (46). What these findings do suggest is that, at least in very young rats, dietary niacin might affect brain function through modulation of the calcium signaling molecule cADPR. Like niacin, brain cADPR in this

![Figure 7](https://example.com/image.png)
study showed an inverse relation with spatial learning ability, and whereas cADPR levels were quickly restored to normal following niacin refeeding, the cognitive benefits associated with the deficiency rapidly disappeared. Nonetheless, many questions remain to be answered before the relation between niacin, CA2+ levels and spatial learning ability is firmly established. Whether changes in cADPR, seen with niacin deficiency and pharmacological supplementation, cause changes in the electrophysiological properties of neurons, remains to be determined.

**Literature Cited**


