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The guinea-pig is a poor animal model for studies of niacin deficiency and presents challenges in any study using purified diets

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The guinea-pig was previously reported as being sensitive to a niacin-deficient (ND), high-protein diet, suggesting that it is a suitable model for the low tryptophan to NAD^+ conversion observed in human subjects. However, these studies were based on growth rates and mortality. The objective of the present study was to determine whether guinea-pigs are suitable for ND studies based on measurements of blood and bone marrow NAD^+. Using a 20 % casein diet, ND decreased blood NAD^+ after 4 weeks, but this parameter returned to normal after 9 weeks of feeding, while bone marrow was decreased by 35 % at this time point. Using a 15 % casein diet, 7 weeks of ND caused 44 and 42 % decreases in blood and bone marrow NAD^+. Using a 10 % casein diet, ND decreased NAD^+ by 32 % in blood and 62 % in bone marrow at 7 weeks. Growth rates were directly related to the dietary tryptophan content, with the lowest growth rates seen with the 10 % casein diet. Changes in guinea-pig NAD^+ are comparable with the rat model at similar levels of dietary tryptophan, while mortality rates were dramatically higher in the guinea-pig model. The present study concludes that mortality in ND guinea-pigs is not indicative of poor tryptophan conversion, but is due to environmental stresses in guinea-pigs that are not observed with rats. We conclude that guinea-pigs are not suitable for research on niacin deficiency and they present challenges for any study requiring purified diets and wire-bottomed cages.

Guinea-pigs: Niacin: Nicotinamide: Nicotinic acid: Pyridine nucleotides: NAD^+

Niacin (vitamin B_3) is required in metabolism for a wealth of redox and ADP-ribosylation reactions. Niacin deficiency in man causes the disease pellagra, which is characterised by unique pathologies such as sun-sensitive dermatitis and schizophrenia-like dementia (Kirkland, 2007). Dietary forms of niacin (including nicotinamide and nicotinic acid) are converted in tissues to the pyridine nucleotides NAD(H) and NADP(H). NAD^+ declines more rapidly than NADH, NADP + or NADPH during niacin deficiency (Fu *et al.* 1989). NAD^+ is involved in energy metabolism, protein modification by mono- and poly-(ADP-ribose) polymerases, and synthesis of cyclic ADP-ribose (Kirkland, 2007).

Recent studies on ADP-ribosylation reactions have illuminated new roles for niacin metabolism in human disease processes. Niacin deficiency impairs poly(ADP-ribose) formation and enhances leukaemogenesis in rat bone marrow (Rawling *et al.* 1995; Boyonoski *et al.* 2002). There is also impaired control of apoptosis and the cell cycle and enhancement of several forms of genomic instability in the deficient state (Spronck *et al.* 2003). Dietary niacin also has a significant impact on skin cancer incidence (Gensler *et al.* 1999; Shah *et al.* 2002). These findings correlate with the human sensitivity to UV light during the development of pellagra. NAD^+ is also required for the deacetylation activity of the sirtuins (Sir2, SirT), which have been shown to play a central role in the lifespan extension caused by energy restriction (Kirkland, 2003). Niacin status may also be a significant factor when these pathways are activated by compounds such as resveratrol, which are generating interest as anti-ageing supplements (Kirkland, 2003). NAD^+ is also required for the formation of cyclic ADP-ribose, which causes Ca release from intracellular stores, and is known to be involved in various aspects of synaptic function. Cyclic ADP-ribose formation is decreased during niacin deficiency and may play a major role in the striking dementia of niacin deficiency in man (Kirkland, 2007). The similarity between pellagrous dementia and schizophrenia suggests that we may be able to extend knowledge of niacin deficiency and brain function to other forms of neurological disturbances.

It is therefore important to characterise suitable animal models to study the effect of dietary niacin depletion. Several animal models have been tested for their response to niacin deficiency, including rats (Burch *et al.* 1955), mice (Spirtes & Alper, 1961) and guinea-pigs (Reid, 1961). One problem with the suitability of rodents in niacin research relates to their ability to convert tryptophan to NAD^+ via the Preiss–Handler (Preiss & Handler, 1958) and Dietrich *et al.* (1966) pathways. Current nutrition guidelines are based on data showing that human subjects convert tryptophan to niacin with an efficiency of 1/60 (Horwitt *et al.* 1981), leading to the use of niacin equivalents (i.e. mg preformed niacin + 1/60 mg tryptophan) to assess dietary intake. In fact, the actual conversion may be even lower if dietary tryptophan status is marginal (Fu *et al.* 1989). In contrast, rats are reported to have a conversion rate of 1/33 (Hankes *et al.*

Abbreviations: ND, niacin deficient; PF, pair fed.

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1948). Mice exhibit more efficient conversion than rats, based on our observations that diets as low as 5 % casein are required to generate a modest niacin deficiency in mice (unpublished results associated with the experiments of Shah *et al.* 2002). Conversion rates are determined mainly by the expression of liver 2-amino-3-carboxymuconic-6-semialdehyde decarboxylase, which diverts tryptophan metabolites away from NAD production (Kirkland, 2007). For example, cats have a high level of expression of this enzyme and an extremely low conversion rate for tryptophan (Kirkland, 2007).

Previous research showed that a 20 % casein, niacin-deficient diet led to complete mortality in guinea-pigs (Reid, 1961), suggesting that they have a poor tryptophan conversion efficiency, and may represent a more accurate model of human metabolism. However, in these previous studies, assessment of guinea-pig susceptibility to niacin deficiency was based on observations of mortality and decreased growth rates, and there have been no subsequent publications on the biochemical characterisation of niacin deficiency in this species. We sought to determine whether depletion of blood and bone marrow NAD^+ supports the idea that the guinea-pig provides a more suitable model of human niacin deficiency than the rat or mouse. Guinea-pigs were maintained on purified diets containing variable levels of tryptophan (casein:gelatin ratio), with and without supplemental niacin. The results of the present study demonstrate that, while changes in blood and bone marrow NAD^+ follow niacin depletion in the guinea-pig, the time course of depletion is variable and the tryptophan content of the diet is an important limiting factor. The modest biochemical changes and high rates of mortality are also problematic.

Materials and methods

Animals

The present study was approved by the Animal Care Committee at the University of Guelph (Guelph, ON, Canada) and adhered to the standards set forth by the Canadian Council on Animal Care. Male Hartley guinea-pigs were purchased from a commercial source (Charles River Canada, St Constant, PQ, Canada) for these experiments. All animals were approximately 6 weeks old when niacin-deficient-diet feeding began. Animals were housed singly in wire-bottomed cages except during weeks 1 and 2 in experiment no. 1, at which time animals were housed in pairs. There was unrestricted access to water, and food provision was as described in the following subsection. Temperature was held constant at 22 ± 2°C, and animals were maintained under a 12 h light–12 h dark condition. To avoid actual animal mortality, health score sheets were used to determine the point at which mortality was likely to follow, and animals were euthanised in keeping with current animal care guidelines. The most objective of these measures was 10 % weight loss from the peak weight on two successive weighings, but moribund behaviour and other health parameters were monitored in these decisions. While these were not technically mortality endpoints, we feel that they are very representative of mortality that would have occurred in these trials, and have continued using mortality as a term to refer to this endpoint. This also simplifies comparisons with older studies.

Diets and procedures

The composition of diets, vitamin and mineral mixes, and the tryptophan content of the diets are shown in Tables 1–4. The experimental models are outlined schematically in Fig. 1.

Experiment no. 1: 20 % casein

Twenty male guinea-pigs of approximately 2 weeks of age were housed in pairs and fed guinea-pig chow (Harlan Teklad, Madison, WI, USA) for the initial 2 weeks post-arrival for acclimatisation. During weeks 3 and 4, all animals were housed singly and were fed the control purified diet containing 20 % casein and added niacin (50 mg/kg). As citrus fruits are part of their natural diet, lemon juice was added to the diet at 1 g/40 g feed to encourage the animals to eat the purified food. At the end of week 4, the animals were divided into two groups. The niacin-deficient group (*n* 10) was fed a diet containing 20 % casein and 0 mg additional nicotinic acid/kg diet, while the pair-fed group (*n* 10) was fed a diet containing 20 % casein and 50 mg nicotinic acid/kg diet (Table 1). The amount of food consumed by each niacin-deficient guinea-pig was determined daily and the same amount of food was given to its pair-fed partner. The pair-feeding strategy was required to maintain equivalent food consumption between the two groups, since niacin deficiency causes anorexia. Blood samples were taken after 4 and 9 weeks on the deficient and pair-fed diets. Animals were euthanised at the 9-week time point and blood and bone marrow samples were collected.

Experiment no. 2: 15 % casein and 10 % gelatin

Eight male guinea-pigs of approximately 4 weeks of age at arrival were housed singly and fed the control purified diet containing 15 % casein and 10 % gelatin with added niacin (50 mg/kg) for 10 d. Gelatin was used as a protein source to

Table 1. Composition of the diets

	Model no. 1: 20 % casein diet (g/kg)	Model no. 2: 15 % casein and 10 % gelatin diet (g/kg)	Model no. 3: 10 % casein and 10 % gelatin diet (g/kg)	Pair-fed control diet (g/kg)
Vitamin-free casein	200·0	150·0	100·0	*
Gelatin	–	100·0	100·0	*
Cerelose	402·88	352·88	402·88	†
Soyabean oil	40·0	40·0	40·0	40·0
Cellulose	150·0	150·0	150·0	150·0
Vitamin mix	30·0	30·0	30·0	30·0
Mineral mix	85·0	85·0	85·0	85·0
L-Arginine	10·0	10·0	10·0	10·0
L-Cysteine	2·5	–	–	*
Methionine	–	2·5	2·5	*
Choline bitartrate	4·0	4·0	4·0	4·0
Phos-C	0·62	0·62	0·62	0·62
Oat bran	50·0	50·0	50·0	50·0
Lemon juice	25	25	25	25
Niacin	–	–	–	0·05

* Each pair-fed control diet contained the same amount of casein, gelatin, cysteine and methionine as its corresponding niacin-deficient diet.

† Each control diet contained 50 mg less cerelose than its corresponding niacin-deficient diet.

Table 2. Composition of vitamin mix

	Vitamin mix (g/kg)
Inositol	16·67
Biotin	0·01
Vitamin B_{12}	1·00
Calcium pantothenate	2·00
Folic acid	0·33
Menadione	1·00
Pyridoxine	0·67
Riboflavin	0·67
Thiamine	0·83
DL-α-Tocopherol	6·67
Vitamin A	1·73
Vitamin D_3	0·13
Sucrose	968·29

limit dietary tryptophan. This diet represents a decrease from 20 to 15 % high-quality protein (casein), with 10 % poor-quality protein (gelatin, essentially no tryptophan) added in an attempt to impair tryptophan utilisation. Lemon juice was added to the diet as described in experiment 1. At the end of the initial 10 d acclimatisation period, animals were divided into two groups. The niacin-deficient group (*n* 4) was fed a diet containing 15 % casein and 10 % gelatin with 0 mg additional niacin/kg diet, while the pair-fed group (*n* 4) was fed a diet containing 15 % casein and 10 % gelatin, with 50 mg added niacin/kg diet (Table 1). Pair feeding was conducted as described in experiment 1. The deficiency period was reduced from 9 to 7 weeks to reduce mortality. We also found that intensive care of the guinea-pigs on a daily basis could help to reduce their loss in appetite, and the development of a depressed attitude that appeared to lead to mortality. This care involved extra handling, daily cleaning of the hair around the face, and direct encouraging of water consumption. Animals were euthanised after 7 weeks on the niacin-deficient and pair-fed diets and blood and bone marrow samples were collected and analysed.

Experiment no. 3: 10 % casein and 10 % gelatin

Twenty-eight male guinea-pigs of approximately 4 weeks of age at arrival were housed singly and fed the control purified diet containing 10 % casein and 10 % gelatin with added nicotinic acid (50 mg/kg) for 10 d. Lemon juice was added to the diet as in the previous models. At the end of the 10 d acclimatisation period, the animals were divided into two groups. The niacin-deficient group (*n* 14) was fed a diet containing 10 % casein and 10 % gelatin and 0 mg additional nicotinic acid/kg diet, while the pair-fed group (*n* 14) was fed a diet containing 10 % casein and 10 % gelatin and 50 mg nicotinic acid/kg diet (Table 1). Pair feeding occurred as described in the previous models. Animals were euthanised after 7 weeks on the niacin-deficient and pair-fed diets and blood and bone marrow samples were collected.

Table 3. Composition of mineral mix

	Mineral mix (g/kg)
Calcium phosphate	298·70
Calcium carbonate	105·84
Sodium phosphate	75·26
Potassium acetate	294·00
Sodium chloride	30·58
Magnesium oxide	58·80
Magnesium sulfate	35·28
Manganese sulfate	7·17
Iron citrate	4·23
Copper sulfate	0·24
Potassium iodide	1·00
Zinc carbonate	1·18
Mo	0·002
Sodium selenate	0·004
Sucrose	87·71

Table 4. Tryptophan content of diets

	Model no. 1: 20 % casein diet (g/kg)	Model no. 2: 15 % casein and 10 % gelatin diet (g/kg)	Model no. 3: 10 % casein and 10 % gelatin diet (g/kg)	Pair-fed control diet (g/kg)	Tryptophan requirements* (g/kg)
Tryptophan (g/kg)	2·2	1·65	1·1	†	1·3

* For maximal growth, as described in Reid (1960).
† Each pair-fed control diet contained the same amount of tryptophan as its corresponding niacin-deficient diet.

Sample collection and preparation

Blood samples were collected at experimental midpoints from ear veins while the animals were under mild sedation with a halothane (Fisher Scientific, Pittsburgh, PA, USA) and N_2O-O_2 mixture. Blood samples were immediately added to cold 1 M-perchloric acid and placed on ice. Proteins were removed by centrifugation (10 min at 14 000 ***g***) and the acidic supernatant fraction was stored at −80°C until NAD^+ analysis. At the completion of each experiment, animals were anaesthetised with a halothane and N_2O-O_2 mixture and decapitation was performed with a guillotine. Arterial blood was collected from euthanised animals following decapitation and processed as described earlier. Bone marrow cells were flushed from femurs with buffered saline and added immediately to cold 1 M-perchloric acid and placed on ice. Proteins were removed by centrifugation (10 min at 14 000 ***g***) and the acidic supernatant fraction was stored at −80°C until NAD^+ analysis.

NAD^+ analysis

Upon thawing, all samples were neutralised to between pH 6·7 and 6·8 with 2 M-KOH and analysed for NAD^+ content via the enzyme cycling of alcohol dehydrogenase (Shah *et al.* 1995).

Statistical analysis

Data are presented as mean values with their standard errors. The Kolmogorov–Smirnov and Shapiro–Wilk tests were used to evaluate normality. Two-tailed independent *t* tests were used

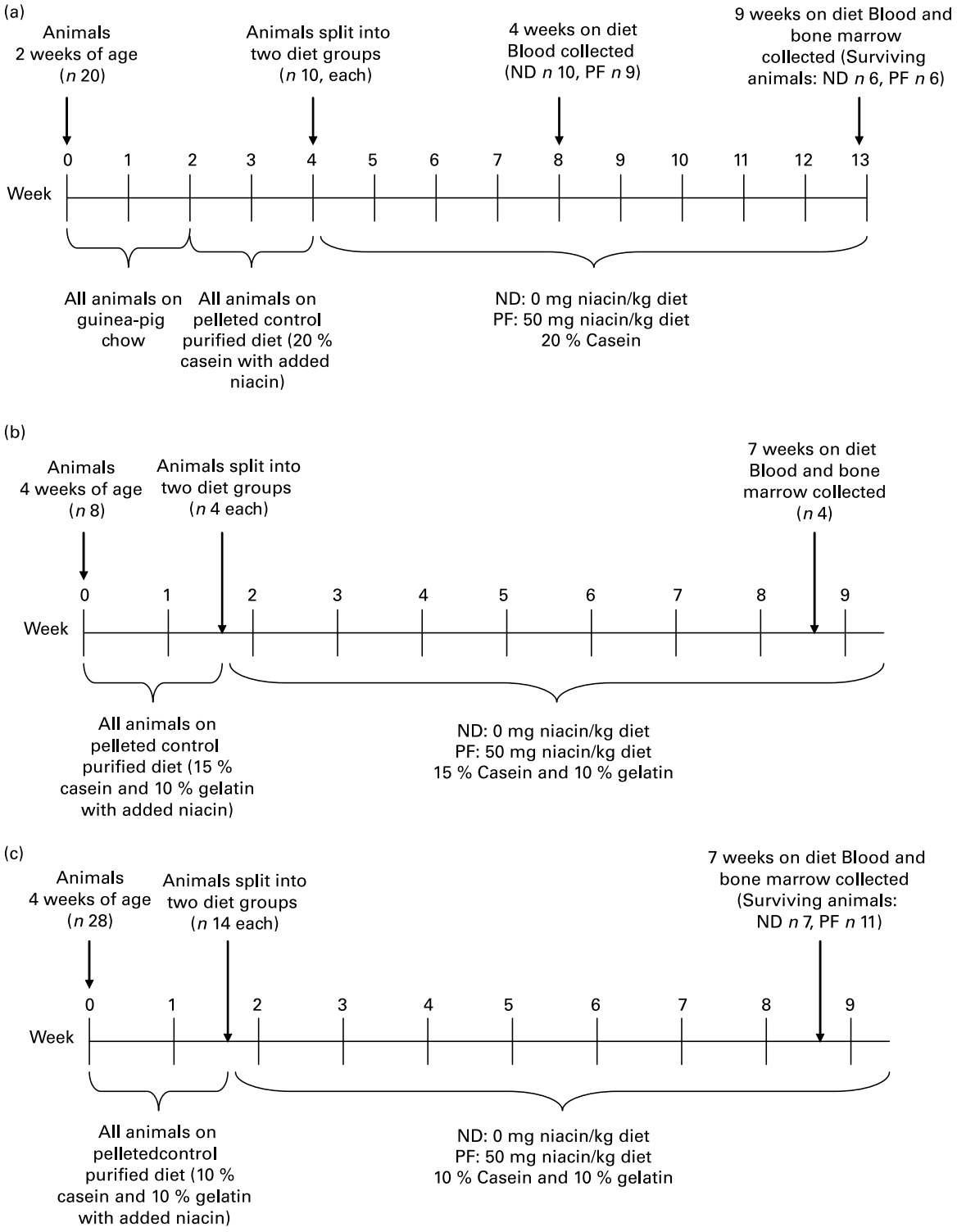

Fig. 1. Schematic representation of the diets and procedures in each experiment. (a) Experiment no. 1, 20 % casein diet; (b) experiment no. 2, 15 % casein and 10 % gelatin diet; (c) experiment no. 3, 10 % casein and 10 % gelatin diet. ND, niacin deficient; PF, pair fed.

to compare levels of NAD^+ between the groups. One-way ANOVA followed by *post hoc* Tukey's test were used to compare growth rates between experiments. All statistical analyses were performed using SPSS version 11.5 for Windows (SPSS Inc., Chicago, IL, USA). A significant P value was set at $\leq 0{\cdot}05$. Data were not transformed before analysis.

Results

Growth

The tryptophan content of the diet had an effect on body weight (Fig. 2). As assessed by one-way ANOVA, there was a significant (F 14·169; $P<0{\cdot}001$) effect of diet on body

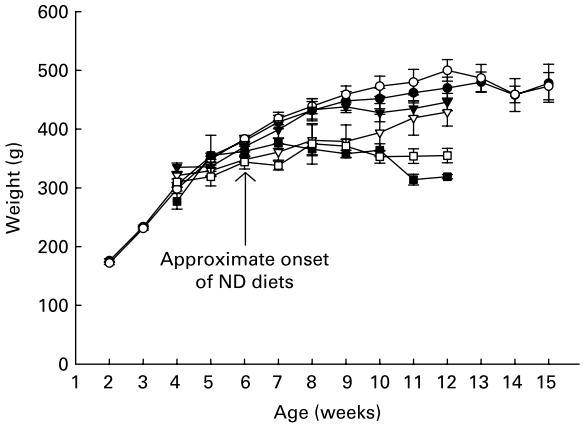

Fig. 2. Body weight over the course of each experiment. (•), Niacin-deficient (ND) 20 % casein diet; (○), pair-fed (PF) 20 % casein diet; (▼), ND 15 % casein diet; (◇), PF 15 % casein diet; (■), ND 10 % casein diet; (□), PF 10 % casein diet. Values are means, with their standard errors represented by vertical bars.

weight. Tukey's *post hoc* test revealed that niacin-deficient and pair-fed guinea-pigs in experiment no. 1 (20 % casein) weighed more than both niacin-deficient ($P<0{\cdot}001$ for both) and pair-fed ($P<0{\cdot}001$ for both) guinea-pigs in experiment no. 3 (10 % casein) after 7 weeks of dietary manipulation. Niacin-deficient guinea-pigs in experiment no. 2 (15 % casein) weighed significantly more than both niacin-deficient ($P=0{\cdot}008$) and pair-fed ($P=0{\cdot}04$) guinea-pigs in experiment no. 3 (10 % casein) after 7 weeks of dietary manipulation. Pair-fed guinea-pigs in experiment no. 2 weighed significantly more than niacin-deficient ($P=0{\cdot}03$) guinea-pigs in experiment no. 3 after 7 weeks of dietary manipulation. Body weights of animals in experiments no. 1 and no. 2 were not significantly different at this time point.

Mortality

In the 20 % casein group at 4 weeks of deficiency, there was a survival rate of 100 % in the niacin-deficient group and 90 % in the pair-fed group. At 9 weeks of deficiency, the survival rate in both groups was 60 %. In the 15 % casein group at 7 weeks of deficiency, there was a survival rate of 100 % in both groups. In the 10 % casein group at 7 weeks of deficiency, there was a survival rate of 50 % in the niacin-deficient group and 78 % in the pair-fed group.

Physical appearance

There was considerable variability in the physical appearance of the niacin-deficient guinea-pigs. Some animals gained weight while others showed variable weight loss. Several animals exhibited intermittent drooling throughout the latter weeks of deficiency, which was associated with reduced feed and water intake. There was a tendency towards diarrhoea, and many animals showed diminished grooming behaviour. Pair-fed guinea-pigs showed lethargic behaviour probably associated with the reduced feed intake and reduced dietary tryptophan content.

Blood NAD^+

In the 20 % casein group at 4 weeks of deficiency, niacin-deficient guinea-pigs showed a non-significant trend for lower blood NAD^+ ($P=0{\cdot}07$). At 9 weeks of deficiency, there was no significant difference between niacin-deficient and pair-fed guinea-pigs (Fig. 3). In the 15 % casein group at 7 weeks of deficiency, niacin-deficient guinea-pigs displayed a significant ($P=0{\cdot}02$) decrease in blood NAD^+ levels by 44 % (Fig. 4). In the 10 % casein group at 7 weeks of deficiency, niacin-deficient guinea-pigs showed a significant ($P<0{\cdot}01$) decrease in blood NAD^+ levels by 32 % (Fig. 4).

Bone marrow NAD^+

In the 20 % casein group at 9 weeks of deficiency, niacin-deficient guinea-pigs showed a significant ($P=0{\cdot}04$) decrease in bone marrow NAD^+ levels by 35 % (Fig. 5). In the 15 % casein group at 7 weeks of deficiency, niacin-deficient guinea-pigs showed a significant ($P=0{\cdot}05$) decrease in bone marrow NAD^+ levels by 42 % (Fig. 5). In the 10 % casein group at 7 weeks of deficiency, niacin-deficient guinea-pigs showed a significant ($P<0{\cdot}001$) decrease in bone marrow NAD^+ levels by 62 % (Fig. 5).

Discussion

Reid (1961) investigated the effect of depletion of dietary niacin on growth and mortality in young Hartley guinea-pigs. However, measurements of NAD^+ levels, which are the *in vivo* biochemical indicator of niacin status (Jacobson & Jacobson, 1993), were not performed at that time. In the last four decades there have been no follow-up studies of niacin deficiency with the guinea-pig, probably because of the adoption of the rat as the preferred animal model in most nutritional investigations. Although we have successfully used the rat in many investigations of niacin insufficiency (Boyonoski *et al.* 2000, 2002; Spronck & Kirkland, 2002; Spronck *et al.* 2003), the study by Reid (1961) suggested that guinea-pigs may be susceptible to niacin deficiency without using

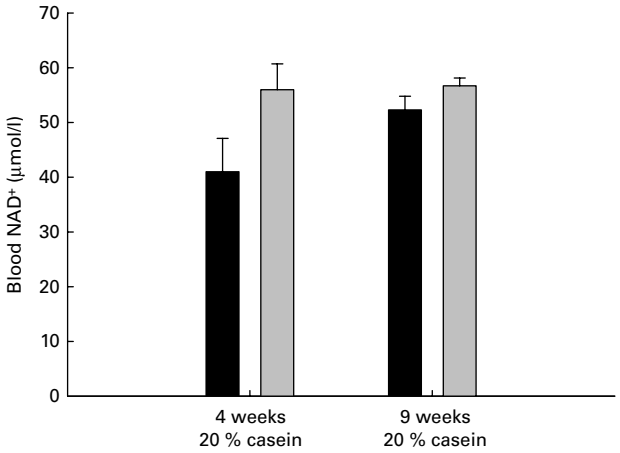

Fig. 3. Blood NAD^+ at 4 and 9 weeks of niacin deficiency in experiment no. 1. (■), Nicacin-deficient animals; (□), pair-fed animals. Values are means, with their standard errors represented by vertical bars.

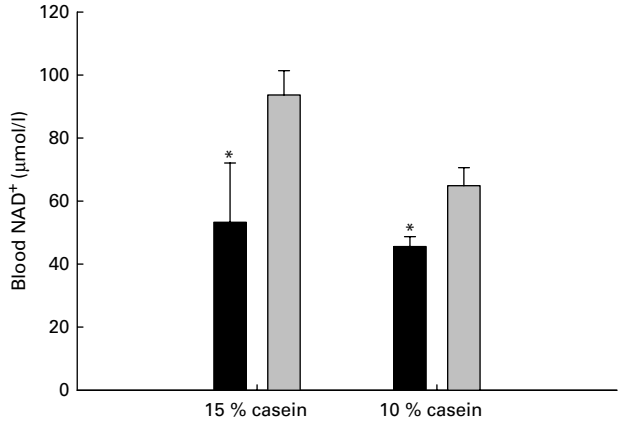

Fig. 4. Blood NAD^+ at 7 weeks of niacin deficiency in experiments no. 1 and no. 2. (■), Nicacin-deficient animals; (▨), pair-fed animals. Values are means, with their standard errors represented by vertical bars.* Mean value was significantly different from that of the pair-fed control group ($P<0{\cdot}05$).

diets that are deficient in tryptophan. This led us to perform a more biochemical characterisation of the model, using blood and bone marrow NAD^+ to determine niacin status. There has been a need to find an animal model with poor tryptophan conversion to more accurately reflect human metabolism. Significant depletion of NAD^+ in rat models that have been developed to this point requires a large reduction in dietary tryptophan, and is also based on the use of juvenile animals. Unfortunately, we have now observed that only modest changes in blood and bone marrow NAD^+ levels result from niacin deficiency in the guinea-pig, with a variable time course of depletion and a strong dependence on the tryptophan content of the diet. Coinciding with our completion of the animal-feeding part of this research, Allegri *et al.* (2003) published a manuscript showing that guinea-pigs express a suitable range of enzymes in the kynurenine pathway to allow tryptophan to niacin conversion, which is in agreement with our findings.

The three diets used in the present study succeeded at depleting NAD^+, with bone marrow NAD^+ levels showing a greater response to niacin deficiency than blood NAD^+ levels, although

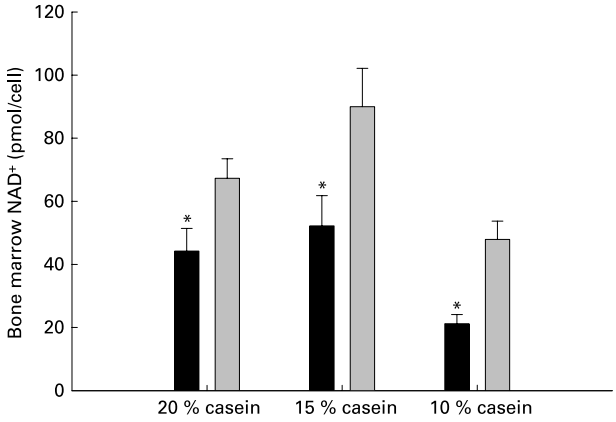

Fig. 5. Bone marrow NAD^+ at the completion of each experiment. (■), Niacin-deficient animals; (▨), pair-fed animals. Values are means, with their standard errors represented by vertical bars.* Mean value was significantly different from that of the pair-fed control group ($P<0{\cdot}05$).

the data obtained from experiments no. 2 and no. 3 are not directly comparable with experiment no. 1 due to the different treatment lengths. The greater sensitivity of bone marrow has been previously observed in rat models (Boyonoski *et al.* 2002). With the 20 % casein diet, at 4 weeks of deficiency, there was a trend towards decreasing blood NAD^+ levels, with niacin-deficient guinea-pigs showing a 27 % decrease compared with pair-fed guinea-pigs. However, by 9 weeks of deficiency, this difference had disappeared. This is not a surprising observation, since we have found that with prolonged niacin deficiency, rats show a recovery from deficiency symptoms (J. B. Kirkland and J. M. Rawling, personal observations) which is probably attributable to an increase in the efficiency of conversion of tryptophan to NAD^+ with age. The initial 20 % casein diet resulted in only a mild decrease in blood and bone marrow NAD^+ levels. To cause a further reduction, the casein component of the diet was reduced to 15 %, and the deficiency period was reduced from 9 to 7 weeks to reduce mortality. At 7 weeks of deficiency with the 15 % casein diet, there was a significant decrease (about 40 %) in both blood and bone marrow NAD^+. Further reduction in the casein content of the diet to 10 % led to a 62 % decrease in bone marrow NAD^+, while blood NAD^+ was not further depressed. Bone marrow NAD^+ was therefore directly related to the tryptophan content of the diet, with NAD^+ levels most affected by the 10 % casein diet. This is consistent with the rat model, where bone marrow NAD^+ is the most labile pool that we have characterised in our rat models of niacin deficiency (Rawling *et al.* 1994; Boyonoski *et al.* 2002). This is probably due to the fact that the bone marrow is the most proliferative tissue in the body, exporting large numbers of cells along with the associated metabolic resources. Bone marrow in the rat model is readily depleted to about 20 % of control NAD^+ (Boyonoski *et al.* 2002), which is about half of the residual NAD^+ that we observed in our most severe guinea-pig model. We have also observed in the rat model that whole-blood NAD^+ depletes readily to a certain point, and then becomes resistant to further change, over time, or in response to more severe diets (Rawling *et al.* 1994). The labile pool in rats appears to be larger, however, where 60 % of whole-blood NAD^+ is readily lost during niacin deficiency in rats (Rawling *et al.* 1994), compared with 30–40 % in our guinea-pig models. Growth rates were also directly related to the tryptophan content of the diet, with the lowest growth rates seen with the 10 % casein diet. Although the tryptophan requirement for maximal growth is 1·3 g/kg diet (Reid & Von Sallmann, 1960), this value assumes ample dietary niacin and a diet composition that guinea-pigs find palatable. The reduced food intake in our studies would have further limited growth.

Although the diets used in the present study were able to produce biochemical changes in blood and bone marrow NAD^+, the magnitude of the observed changes are less than those observed in studies with rats. In young Long-Evans rats, we observed an 80 % decrease in bone marrow NAD^+ (Spronck & Kirkland, 2002; Spronck *et al.* 2003) and a 60 % decrease in blood NAD^+ (Rawling *et al.* 1996) after 3 weeks of niacin deficiency. With rats, we observe obvious signs of niacin deficiency within 2–3 weeks, such as porphoryn staining around the nose and mouth and diarrhoea. These signs were not observed with guinea-pigs until much later in the deficiency period, and only in a proportion of the deficient animals. With rats, we are able to use diets

containing as low as 7 % casein without experiencing significant mortality in the niacin-deficient group (Rawling *et al.* 1994; Boyonoski *et al.* 2002). With guinea-pigs, however, we observed a mortality rate of approximately 50 % with a 10 % casein diet. These observations suggest that in order to reduce the blood and bone marrow NAD^+ in guinea-pigs to the same levels seen in rats, the casein content of the diet would have to be reduced further which would undoubtedly increase the rate of mortality.

The purpose of the present study was to assess whether the guinea-pig is a suitable animal for niacin-deficiency studies, unlike the original experiment which sought to determine the niacin requirements of the guinea-pig (Reid, 1961). Although we duplicated several features of the original investigation, many modifications were required to make it suitable for this purpose. First, we made changes to the semi-synthetic diet developed by Reid & Briggs (1953). In experiments no. 2 and no. 3, gelatin was added as a protein source to limit the tryptophan content. The carbohydrate source was changed to cerelose because of the potential niacin content of maize starch and the tendency of large amounts of fructose (from sucrose) to disrupt liver lipid metabolism. Soyabean oil was used instead of maize oil due to its higher content of essential fatty acids. Second, although we initially attempted to use weanling guinea-pigs in these experiments, these animals showed a rapid and dramatic loss of body weight. This required us to change to a slightly older animal model in order to avoid an excessive level of mortality. Using weanling guinea-pigs fed a 20 % casein diet, Reid (1961) observed a mortality rate of 100 %, which is not conducive to chronic studies of niacin deficiency. Using the young guinea-pig model we were able to decrease the mortality rate below 50 %, even when using a very low level of dietary tryptophan. Third, the control guinea-pigs were pair fed, which is an absolute requirement of studies of niacin deficiency due to the development of anorexia (Kirkland & Rawling, 2001). The control animals in the original study were fed *ad libitum* (Reid, 1961), which would have resulted in an unequal delivery of macro- and micronutrients and complicated a direct comparison between the groups. The lack of pair feeding in the original study limits the use of mortality data in drawing direct correlations to niacin deficiency from that model.

By switching to a pair-feeding model, we demonstrated that there was an elevated rate of mortality and apparent stress response in the pair-fed control group, which is highly problematic. Animals in the pair-fed control group showed extreme lethargy and lack of motivation to engage in normal feeding behaviour. We feel that the most plausible explanation for this behaviour is the change in husbandry required by the pair feeding model. Although it is not explicitly stated, it is likely that the animals in the original study were pair or group housed in solid-bottomed cages, since food intake was not determined and the animals were fed *ad libitum* (Reid, 1961). In contrast, our guinea-pigs were individually housed in wire-bottomed cages in order to accurately measure food intake and to reduce coprophagy and limit intake of NAD^+ formed by gut micro-organisms. Additionally, there is evidence that guinea-pigs are healthier when consuming a commercial diet rather than a semi-purified diet (Reid & Mickelsen, 1963). The necessity of using a semi-purified diet in niacin-deficiency studies may have contributed to the poor health and behaviour of the control group, and would also have a confounding effect on the health of the niacin-deficient group. The housing and diet requirements for niacin-deficiency studies may therefore be challenges for the guinea-pig model in comparison with the rat, which adapts quite well to the same environmental situation. For these reasons, there will be challenges in any studies with guinea-pigs requiring single housing in wire-bottomed cages and with the use of purified or semi-purified diets. These effects can be countered to some degree by intensive animal care, including extra handling and washing of fur and encouragement of water consumption.

The changes in the blood and bone marrow NAD^+ levels in the niacin-deficient guinea-pig model are modest when compared with the rat. The changes in both species would probably be comparable if the guinea-pig were able to tolerate a reduction in casein below 10 %, but the issue of mortality would seriously confound such an investigation. We have also found that the environmental requirements of niacin-deficiency studies create problems with the guinea-pig control group, a scenario that is not observed with the rat. We therefore conclude that the guinea-pig is not a suitable animal for investigations of niacin deficiency, and future studies on NAD involvement in neural function, genomic stability and longevity may require the development of transgenic animals with impaired conversion of tryptophan to niacin. This should be possible through over-expression of liver 2-amino-3-carboxymuconic-6-semialdehyde decarboxylase.

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