British Journal of Nutrition (2004), **91**, 741–747
DOI: 10.1079/BJN20041104

Dietary repletion can replenish reduced T cell subset numbers and lymphoid organ weight in zinc-deficient and energy-restricted rats

Heather J. Hosea[1], Edward S. Rector[2] and Carla G. Taylor[1]*

Departments of [1]Human Nutritional Sciences and
[2]Immunology, University of Manitoba, Winnipeg, Man. R3T 2N2, Canada

(Received 15 July 2003 – Revised 29 November 2003 – Accepted 22 January 2004)

The objective of the present study was to investigate the time course for recovery of lymphoid tissue and T cell subset numbers when Zn-deficient (ZD) or energy-restricted (ER) rats were repleted with control diet; in a second experiment, the link between the stress axis and lymphoid organs was explored. During the deficiency phase, rats were fed a ZD (< 1 mg Zn/kg) or control diet (30 mg Zn/kg, nutritionally complete) either as pair-fed controls (ER) or *ad libitum*-fed controls (CTL) for 3 weeks. During the repletion phase, all rats were fed control diet *ad libitum* for 3, 7 or 23 d. After the deficiency phase, ZD and ER had lower T cell subset numbers in the thymus compared with CTL, and ZD had reduced T cell subset numbers in the spleen compared with both ER and CTL. T cell subset numbers and lymphoid organ weights recovered from dietary Zn deficiency and energy restriction by 7 d of repletion (except 23 d for thymus weight in ZD), while body weight required more than 23 d for recovery. At the end of the deficiency phase, ZD and ER had higher circulating corticosterone concentrations compared with CTL; plasma TNFα was not detectable and there were no differences in plasma haptoglobin, an acute-phase protein. In conclusion, Zn deficiency and energy restriction elevated circulating corticosterone and reduced T cell subset numbers in the thymus and spleen of growing rats. Repletion with a nutritionally complete diet allowed recovery of T cell subset numbers and lymphoid organ weight.

T cell subsets: Zinc deficiency: Energy restriction: Repletion: Rats

All cells in the body require nutrients to function properly, and a deficiency in any of these required nutrients can cause immune function to be compromised. Both dietary Zn deficiency and energy malnutrition in mice are characterized by reduced growth, atrophy of lymphoid tissue, reduced lymphocyte numbers and increased susceptibility to infection (Woodward, 1998; Fraker *et al.* 2000). High concentrations of corticosterone have been shown to increase apoptosis *in vitro* in lymphocytes from rats (Hughes & Cidlowski, 1998); the decline in the proportion of $CD4^+CD8^+$ pre-T cells in the thymus of Zn-deficient mice has been attributed to greater apoptosis in this T cell subset due to elevated circulating corticosterone concentrations (King *et al.* 2002). It has been suggested that dietary Zn deficiency stimulates the hypothalamus–pituitary–adrenal stress axis, leading to increased plasma corticosterone levels: this may explain the lymphopaenia and thymic atrophy associated with dietary deficiencies (Fraker *et al.* 1995). TNFα stimulates the hypothalamus–pituitary–adrenal axis through several intermediates, including adrenocorticotropic hormone (ACTH), which increases the release of corticosterone from the adrenal glands (Steel & Whitehead, 1994). TNFα and corticosterone act on the liver to increase the induction of acute-phase proteins such as haptoglobin (Steel & Whitehead, 1994). Thus, the stress axis involves interactions among many components, including ACTH, corticosterone, TNFα and haptoglobin.

There has been a considerable amount of research on the effects of dietary Zn deficiency and protein–energy malnutrition on immune function; however, the role of nutrients in the recovery of the immune system from nutritional deprivation is important for development of nutritional therapies. Thus, our first objective was to investigate the time course for recovery of lymphoid tissue and T cell subset numbers when Zn-deficient or energy-restricted rats were repleted with a nutritionally complete control diet. In a second experiment, the potential link between markers of the stress axis (ACTH, corticosterone, TNFα and haptoglobin) and atrophy of the lymphoid organs in the dietary deficiencies was explored. To separate the effects of Zn deficiency from energy malnutrition, an energy-restricted group (pair-fed to the Zn-deficient rats) was included. Three time points were chosen for dietary

Abbreviations: ACTH, adrenocorticotropic hormone; CTL, *ad libitum*-fed control group; ER, energy-restricted group; TCR, T cell receptor; ZD, zinc-deficient group.

* **Corresponding author:** Dr Carla G. Taylor, fax + 1 204 474 8079, email ctaylor@ms.umanitoba.ca

repletion: 3 d to identify any rapid changes (lymphocytes proliferate quickly), 7 d based on recovery of thymus and spleen weights in adult Zn-deficient or protein-malnourished mice (Fraker *et al.* 1978; Taylor *et al.* 1997) and 23 d based on the timeframe for murine T lymphocyte maturation (Sharon, 1998). The growing rat model represents an age group that is at risk for Zn deficiency and energy malnutrition (Briefel *et al.* 2000). The commercial availability of antibodies for the rat presents new opportunities to investigate immune function using rat models in which nutritional interventions have been extensively characterized.

Experimental methods

Animals and diets

Expt 1. Ninety-eight 3-week-old male Sprague–Dawley rats (Charles River Laboratories, St Constant, Que., Canada) were acclimatized for 5 d; they were then randomly assigned to the baseline group (*n* 8) or were fed a Zn-deficient diet *ad libitum* (ZD group; <1 mg Zn/kg, *n* 30), or were fed a nutritionally complete control diet (30 mg Zn/kg) either *ad libitum* (CTL; *n* 30) or pair-fed to the ZD group (ER; energy restricted; *n* 30) for 3 weeks (deficiency phase). At the end of the deficiency phase, eight animals per dietary treatment group were killed and the remaining twenty-two rats per group began the repletion phase. During the repletion phase, rats were fed the control diet for 3 (eight per group), 7 (eight per group) or 23 (six per group) d. The experimental diets, containing egg albumin, additional biotin (2 mg/kg diet) and potassium phosphate (5·4 g/kg diet for the growth formulation), have been previously described by Lepage *et al.* (1999). The Zn content of the diets was verified by atomic absorption analysis. Care was taken to avoid Zn recycling and contamination by housing the rats in stainless-steel hanging cages with mesh bottoms and by providing distilled water in plastic bottles with stainless-steel sipper tubes. The rats were maintained in an environment of controlled temperature (21–23°C), humidity (55 %) and light cycle (14 h light–10 h dark). Body weights were determined weekly and feed intake was determined daily. Animal care was provided in accordance with a protocol approved by the Local Animal Care Committee (University of Manitoba).

Expt 2. To investigate the response of the stress axis, the animals and diets were as described earlier, but confined to the deficiency phase only. The rats were handled by the same investigator each day during the acclimation and experimental periods to minimize the effects of handling stress before phlebotomy (Shipp & Woodward, 1998).

Tissue collection

At baseline, after the 3-week deficiency phase, and after 3, 7 or 23 d of repletion, rats were killed by CO_2 asphyxiation and cervical dislocation between 08.00 and 09.00 hours. Trunk blood was collected in EDTA vacutainer tubes (Becton Dickinson, Franklin Lakes, NJ, USA) or blood collection tubes, centrifuged to obtain plasma or serum, and stored at -80°C until analysis. Thymus and spleen were removed aseptically, weighed and processed immediately.

Zinc analysis

After obtaining wet and dry weights, thymus, spleen and diet samples were wet-ashed using trace-element grade HNO_3. After appropriate dilution of digests, Zn concentration was determined by atomic absorption spectroscopy using a Spectra AA-30 spectrophotometer (Varian Canada, Georgetown, Ont., Canada). Quality control was monitored using bovine liver standard reference material 1577b (US Department of Commerce, National Institute of Standards and Technology, Gaithersburg, MD, USA).

Determination of T-lymphocyte subpopulations

Single-cell suspensions were obtained by gently suspending the thymus and spleen in PBS supplemented with bovine fetal calf serum (10 ml/l; Gibco, Grand Island, NY, USA) using a glass–glass tissue grinder (Koontes, Vineland, NJ, USA). Cells remained intact as verified by Trypan Blue exclusion. Single-cell suspensions from thymus and spleen (1×10^6 mononuclear cells; splenocytes separated by Lympholyte-Rat (Cedarlane, Hornby, Ont., Canada)) were incubated with monoclonal antibodies (obtained from BD Pharminigen, Mississauga, Ont., Canada) for T cell receptor (TCR)αβ (PE label, R73 clone), CD4 (PC5 label, OX-35 clone) and CD8 (FITC label, G28 clone). Flow-Count™ Fluorospheres (Beckman Coulter, Mississauga, Ont., Canada) were added to obtain absolute counts. Flow cytometry analysis was performed on a Beckman Coulter EPICS ALTRA (Beckman Coulter) high-speed cell sorter using the EXPO32 MultiCOMP software provided with the instrument. Forward-angle *v.* side-scatter histograms were used to gate for single cells. Fluorochrome-isotype matched controls were prepared to assess autofluorescence and non-specific binding, while samples stained individually with a single fluorochrome were employed to adjust colour compensation. The data were collected in 'listmode' format and the subsequent analysis based on 10 000 cells satisfying the light-scatter gating criteria. Absolute counts of T cell subsets were calculated based on the total number of cells counted, the total number of fluorospheres counted and the concentration of Flow-Count™ Fluorospheres (Beckman Coulter). Cell counts were corrected for the weight of the thymus or spleen used to prepare the cell suspensions.

Biochemical measurements

Corticosterone and ACTH concentrations were determined by radioimmunoassay kits (ICN Biomedicals Inc., Costa Mesa, CA, USA). Plasma TNFα and haptoglobin concentrations were determined using an ELISA (Alpco Diagnostics, Whidham, NH, USA; detection limit 15·6 pg/ml) and colorimetric assay (Tridelta Development, Wicklow, Republic of Ireland) respectively. Samples were analysed in duplicate and agreement was >85 % ($r^2 > 0{\cdot}99$ for standard curves).

Statistical analyses

Differences among dietary treatment groups and over time were analysed by one-way ANOVA using the general linear models procedure (Statistical Analysis Systems software release 8.2; SAS Institute, Cary, NC, USA). When necessary, data were normalized by log transformation for statistical analyses, but non-transformed means are reported. Significant differences among mean values were determined using Duncan's new multiple range test. Differences were considered significant at $P<0{\cdot}05$.

Results

ZD consumed less feed per d (Table 1) and weighed 49 % less than CTL at the end of the deficiency phase (Fig. 1(a)). For ZD and ER there was no difference in feed consumption during the deficiency phase, but ZD weighed 14 % less than ER. The high feed efficiency ratio reveals that ZD needed to consume more feed to gain the same weight as the other groups during the deficiency phase. During the first 3 d of repletion with the control diet, the feed intake of ZD and ER rats increased 88 and 123 % respectively, while CTL increased feed intake by only 15 %. During repletion, ZD and ER also had a greater rate of weight gain than CTL. Throughout the repletion phase ZD and ER had lower feed efficiency ratios than CTL, indicating that ZD and ER were able to gain more weight with less feed compared with CTL during the repletion phase.

The lymphoid organ:body weight ratios were highest at baseline and decreased with age in all groups (Fig. 1(c and e)). There were no differences among groups in either thymus:body weight or spleen:body weight ratios at the end of the deficiency phase. Both thymus and spleen weights were lower in ZD and ER compared with CTL at the end of the deficiency phase, and ZD were lower than ER (Fig. 1(b and d)). During the deficiency phase, the lymphoid organ weights of ZD decreased 20 % compared with baseline, and there was no change in ER, while thymus and spleen weights of CTL increased 81 and 78 % respectively compared with baseline. In ZD, spleen weight recovered to CTL values by 7 d and thymus weight recovered by 23 d of repletion. In ER both thymus and spleen weights recovered to control levels by 7 d of repletion. There were no differences in thymus or spleen Zn concentrations throughout the study (results not shown).

There was no difference among dietary treatment groups in the absolute number per organ of the most immature thymic cells ($TCR\alpha\beta^{-}CD4^{-}CD8^{-}$; results not shown). At the end of the deficiency phase, ZD and ER rats had 35–52 % fewer thymic pre-T cells ($TCR\alpha\beta^{-}CD4^{+}CD8^{+}$ and $TCR\alpha\beta^{+}CD4^{+}CD8^{+}$) compared with CTL, and ZD had fewer thymic $TCR\alpha\beta^{+}CD4^{+}CD8^{-}$ (helper) cells compared with CTL (Fig. 2(a, b, c and d)). There were no differences in thymic T cell subset numbers among dietary treatment groups after 7 d of repletion. ZD rats had 40–63 % fewer splenic $TCR\alpha\beta^{+}CD4^{+}CD8^{-}$ (helper) and $TCR\alpha\beta^{+}CD4^{-}CD8^{+}$ (cytolytic) cells (Fig. 2(e and f)) compared with both ER and CTL at the end of the deficiency phase, but not at any other time point. ER had 39 % fewer splenic helper T cells compared with CTL at the end of the deficiency phase, but recovered to CTL levels after 3 d of repletion. There were no differences in the number of T cell subsets among dietary treatment groups when corrected for lymphoid organ weight (results not shown).

Adrenal gland weight:body weight ratios were highest at baseline (Fig. 3). At the end of the deficiency phase, ZD and ER had 65 and 34 % respectively higher adrenal gland weight:body weight ratios compared with CTL, and ZD was 23 % higher than ER. The adrenal gland

Table 1. Effects of zinc deficiency and energy restriction followed by repletion on feed efficiency in growing rats‡
(Mean values with their standard errors)

			Dietary group					
			ZD		ER		CTL	
	Time	*n*	Mean	SE	Mean	SE	Mean	SE
Feed intake (g/d)	Deficiency	8	$9{\cdot}8^{b*}$	0·3	$10{\cdot}2^{b*}$	0·3	$20{\cdot}3^{b}$	0·4
	3 d repletion	8	$18{\cdot}4^{a*}$	0·9	$22{\cdot}7^{a}$	0·6	$23{\cdot}3^{a}$	0·7
	7 d repletion	8	$20{\cdot}0^{a*}$†	0·7	$23{\cdot}0^{a}$	0·9	$23{\cdot}9^{a}$	1·2
	23 d repletion	6	$20{\cdot}2^{a}$	0·8	$22{\cdot}1^{a}$	0·7	$22{\cdot}5^{ab}$	1·0
Weight gain (g/kg body weight per d)§	Deficiency	8	12^{c}	1	19^{a}	2	42^{a}	1
	3 d repletion	8	68^{a*}†	3	82^{a*}	3	26^{b}	1
	7 d repletion	8	61^{a}	3	62^{b*}	1	23^{b}	2
	23 d repletion	6	35^{b*}	1	32^{c*}	1	16^{c}	1
Feed efficiency ratio‖	Deficiency	8	26^{a*}†	1	21^{b}	1	19^{c}	1
	3 d repletion	8	$1{\cdot}7^{b*}$	0·1	$1{\cdot}5^{d*}$	0·1	$3{\cdot}1^{b}$	0·2
	7 d repletion	8	$1{\cdot}8^{b*}$	0·1	$1{\cdot}8^{c*}$	0·1	$3{\cdot}9^{ab}$	0·8
	23 d repletion	6	$2{\cdot}5^{a*}$	0·1	$2{\cdot}6^{a*}$	0·1	$4{\cdot}3^{a}$	0·3

ZD, zinc-deficient group; ER, energy-restricted group; CTL, control group.
[a,b,c]Mean values within a column for each variable with unlike superscript letters were significantly different: $P<0{\cdot}05$.
Mean values were significantly different from those of CTL at each time point: *$P<0{\cdot}05$.
Mean values were significantly different from those of ER at each time point: †$P<0{\cdot}05$.
‡ For details of diets and procedures, see p. 742.
§ Rate of weight gain = ((final body weight (g) − initial body weight (g)/average weight (g))/ days (*n*).
‖ Feed efficiency ratio = total food intake (g)/total weight gained (g).

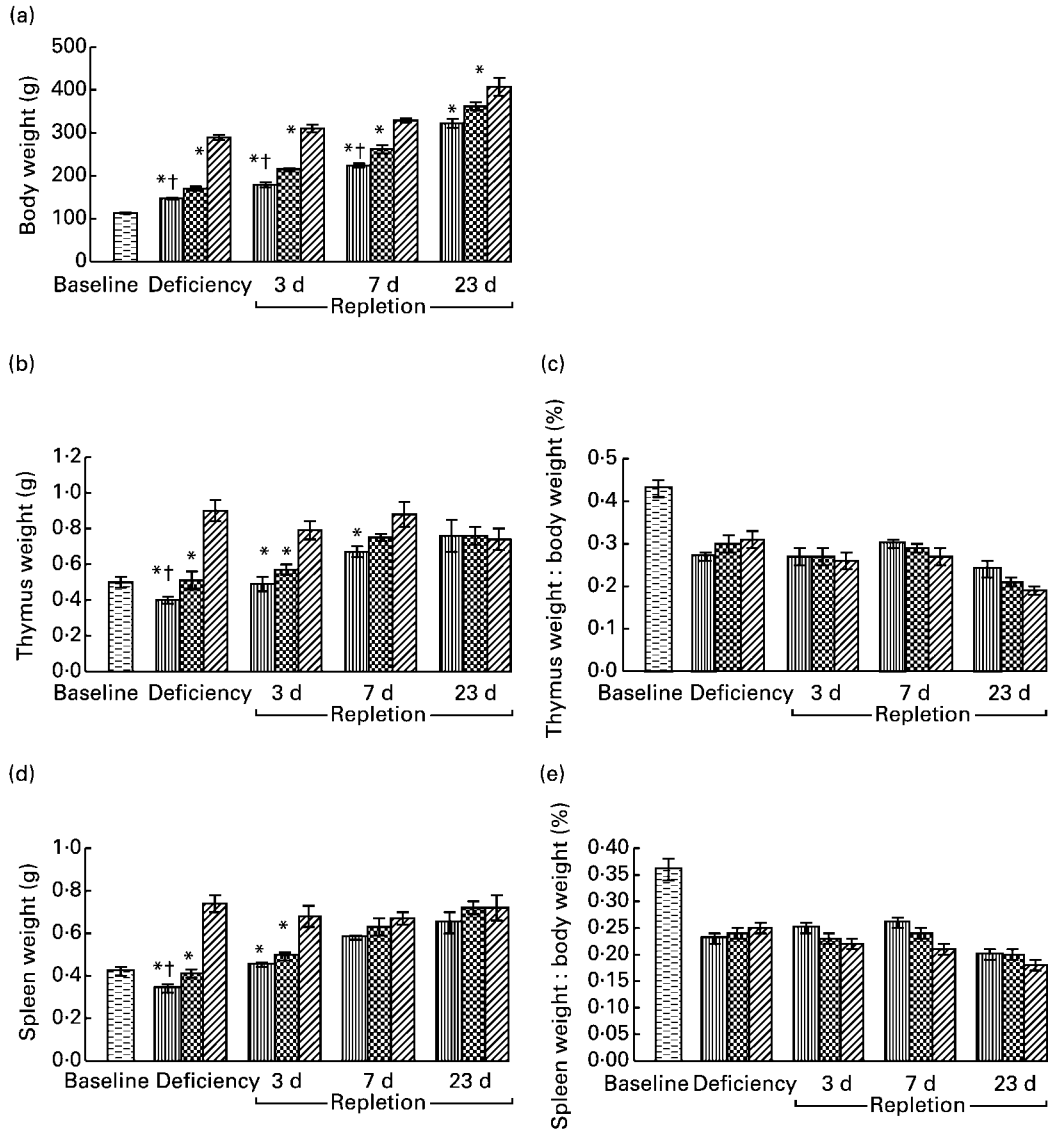

Fig. 1. Effects of zinc deficiency, energy restriction and repletion on body, thymus and spleen weights and ratios in growing rats. ▥, Zinc-deficient group; ▦, energy-restricted group; ▨, control group. For details of diets and procedures, see p. 742. Values are means with their standard errors shown by vertical bars (baseline, deficiency, 3 d and 7 d repletion: eight rats per group; 23 d repletion: six rats per group). Mean values were significantly different from those of the control group at each time point: *$P<0{\cdot}05$. Mean values were significantly different from those of the energy-restricted group at each time point: †$P<0{\cdot}05$.

weight:body weight ratio was not different from CTL after 3 d of repletion for ER and after 7 d of repletion for ZD.

Markers of the stress axis were determined in a separate group of rats where special care was taken to minimize environmental stressors. ZD and ER had 338–527 % higher serum corticosterone concentrations compared with CTL (Table 2). There were no differences among dietary treatment groups for plasma ACTH and haptoglobin concentrations, and TNFα was not detectable in the plasma (results not shown).

Discussion

Both Zn deficiency and energy restriction in growing rats elevated circulating corticosterone and reduced thymic pre-T cell numbers, while Zn-deficient rats had fewer splenic helper and cytolytic T cells compared with energy-restricted and control rats. In addition, ZD had fewer thymic helper T cells and ER had fewer splenic helper T cells compared with CTL at the end of the deficiency phase. T cell subset numbers and lymphoid organ weights recovered from dietary Zn deficiency and energy restriction after 7 d of repletion with a nutritionally complete diet, while body weight required >23 d to catch up to CTL in growing rats. Spleen weight and spleen T cell subset numbers recovered faster than the same variables in the thymus of ZD and ER rats. There appears to be a priority for recovery of lymphoid organs before body weight enabling the body to produce more T lymphocytes and release them into circulation for immune defence while nutritional recovery is in progress.

In growing rats, reduced dietary intake of Zn or energy resulted in stunting malnutrition (Fig. 1). As expected, thymus and spleen weights were lower in ZD and ER at

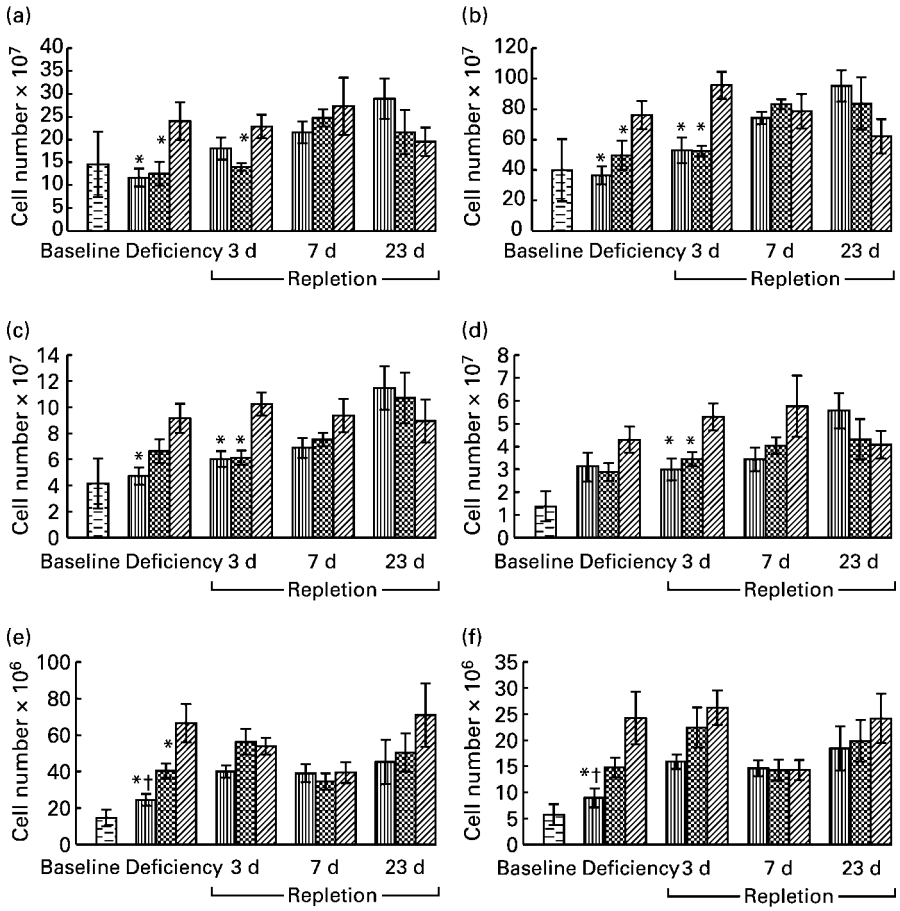

Fig. 2. Effects of zinc deficiency, energy restriction and repletion on thymus and spleen T cell subset numbers (per organ) in growing rats. ▥, Zinc-deficient group; ▦, energy-restricted group; ▨, control group. For details of diets and procedures, see p. 742. (a), Thymus $TCR\alpha\beta^{-}CD4^{+}CD8^{+}$; (b), thymus $TCR\alpha\beta^{+}CD4^{+}CD8^{+}$; (c), thymus $TCR\alpha\beta^{+}CD4^{+}CD8^{-}$; (d), thymus $TCR\alpha\beta^{+}CD4^{-}CD8^{+}$; (e), spleen $TCR\alpha\beta^{+}CD4^{+}CD8^{-}$; (f), spleen $TCR\alpha\beta^{+}CD4^{-}CD8^{+}$. Cells were triple-labelled to identify T cells at various stages of maturation in the thymus. T cell maturation from immature to mature is as follows: $TCR\alpha\beta^{-}CD4^{-}CD8^{-}$ (pro-T cell) → $TCR\alpha\beta^{-}CD4^{+}CD8^{+}$ (pre-T cell) → $TCR\alpha\beta^{+}CD4^{+}CD8^{+}$ (pre-T cell) → $TCR\alpha\beta^{+}CD4^{+}CD8^{-}$ (helper T cell) or $TCR\alpha\beta^{+}CD4^{-}CD8^{+}$ (cytolytic T cell). Values are means with their standard errors shown by vertical bars (baseline, deficiency, 3 d and 7 d repletion: eight rats per group; 23 d repletion: six rats per group). Mean values were significantly different from those of the control group at each time point: $^{*}P<0{\cdot}05$. Mean value was significantly different from that of the energy-restricted group at each time point: $\dagger P<0{\cdot}05$.

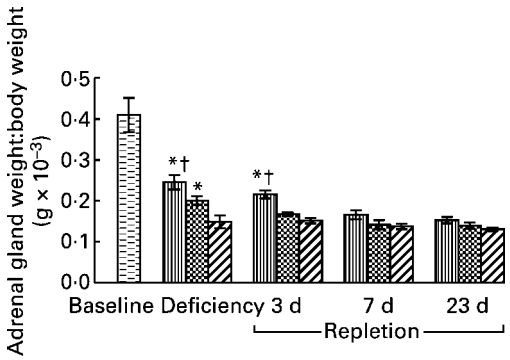

Fig. 3. Effects of zinc deficiency, energy restriction and repletion on adrenal gland weight:body weight ratios in growing rats. ▥, Zinc-deficient group; ▦, energy-restricted group; ▨, control group. For details of diets and procedures, see. p. 742. Values are means with their standard errors shown by vertical bars (baseline, deficiency, 3 d and 7 d repletion: eight rats per group; 23 d repletion: six rats per group). Mean values were significantly different from those of the control group at each time point: $^{*}P<0{\cdot}05$. Mean value was significantly different from that of the energy-restricted group at each time point: $\dagger P<0{\cdot}05$.

the end of the deficiency phase; this is similar to the adult mouse model (Fraker *et al.* 1977, 1982; Cook-Mills & Fraker, 1993). Lymphoid organ atrophy relative to body weight is present in the adult Zn-deficient mouse (Fraker *et al.* 1977; Cook-Mills & Fraker, 1993; Lepage *et al.* 1999), a model of wasting malnutrition. However, in growing Zn-deficient rats, a model of stunting malnutrition, lymphoid organ weights relative to body weight were not different from ER or CTL (Fig. 2; Giugliano & Millward, 1984). In growing Zn-deficient rats, the substantial (70 %) reduction of femur Zn concentrations (Hosea *et al.* 2003) indicates the severity of the Zn deficiency; however, there was no thymic or splenic atrophy relative to body weight (Fig. 1), which is used as an indicator of severe immunodeficiency (Fraker *et al.* 2000). Others have reported reduced lymphocyte numbers in rodent models of Zn deficiency and protein–energy malnutrition and there was a greater reduction of lymphocyte numbers in presence of wasting malnutrition compared with stunting malnutrition (Bises *et al.* 1987; Cook-Mills & Fraker, 1993). In the present study using flow cytometry and

Table 2. Effects of zinc deficiency and energy restriction on circulating markers of the stress axis† (Mean values with their standard errors for nine rats per group)

	Dietary group					
	ZD		ER		CTL	
	Mean	SE	Mean	SE	Mean	SE
Serum corticosterone (nmol/l)	329*	79	508*	131	9·46	0·98
Plasma ACTH (pg/ml)	605	110	727	96	548	91
Plasma haptoglobin (mg/ml)	0·69	0·05	0·65	0·05	0·52	0·06

ZD, zinc-deficient group; ER, energy-restricted group; CTL, control group; ACTH, adrenocorticotropic hormone.
Mean values were significantly different from that of CTL: *$P<0·05$.
† For details of diets and procedures, see p. 000.

Flow Count™ Fluorospheres (Beckman Coulter), ZD and ER had lower thymus and spleen cell numbers (sum of subsets) compared with CTL, and ZD had fewer spleen TCR$\alpha\beta^+$ cell numbers compared with ER at the end of the deficiency phase (Fig. 2). Cell numbers responded rapidly to repletion, recovering to CTL levels by 7 d in ER and ZD. T cell maturation takes approximately 3 weeks in mice (Sharon, 1998); thus, the increase in cell numbers reflects the ability of existing cells to replicate. Thymus and spleen cell numbers per g tissue were not different between ZD and ER in growing rats (present study) or in adult mice (Lepage *et al.* 1999). Although ZD and ER rats maintained lymphoid cell numbers proportional to tissue weight and lymphoid organ weights proportional to body weight, the lower number of lymphocytes per animal may reduce the T cell repertoire and may contribute to the greater susceptibility of deficient animals to infection (Woodward, 2003).

In the present study, serum corticosterone concentrations were elevated in both ZD and ER compared with CTL (Table 2). Others have reported that 3–6-week-old mice fed a Zn-deficient diet for 19–31 d have approximately 200 % higher plasma corticosterone concentrations (determined by spectrofluorometric method) and a 33 % greater adrenal gland weight:body weight ratio compared with mice fed the Zn-adequate diet *ad libitum* (DePasquale-Jardieu & Fraker, 1979). When male Sprague–Dawley rats (150–160 g) were fed a Zn-deficient diet for 40 d, serum corticosterone concentrations (determined by a RIA kit (ICN Biomedicals Inc.)) were 122 % higher than rats fed the control diet *ad libitum* (Nobili *et al.* 1997). Neither of these studies reported the circulating corticosterone concentrations in an energy-restricted group. Cytokine TNFα and acute-phase protein haptoglobin have been associated with corticosterone concentrations during inflammation (Steel & Whitehead, 1994), but plasma TNFα was not detected and haptoglobin was unchanged by dietary treatment indicating the absence of infection in these animals.

One of the hypotheses for the low lymphoid organ cell numbers in Zn deficiency is that elevated corticosterone promotes apoptosis in lymphoid cells (Fraker *et al.* 1995). King *et al.* (2002) have reported elevated serum corticosterone and higher proportions of apoptosis in pre-T cells and a lower percentage of these cells in the thymus of Zn-deficient mice, whereas Moore *et al.* (2001) found no changes in proportions of thymic T cells in Zn-deficient mice. Both studies reported a similar reduction in serum Zn; however, there was no weight loss in the study of Moore *et al.* (2001), while Zn-deficient mice in the study of King *et al.* (2002) weighed 29 % less than control mice. Although feed intake was similar (King *et al.* 2002), the specific role of Zn deficiency *v.* the role of weight loss was not addressed. In the growing rat model of Zn deficiency, we found no changes in the proportions of T cell subsets in the thymus and spleen, except for a higher proportion of thymic cytolytic T cells in Zn-deficient rats (Hosea *et al.* 2003). In the present study, ZD and ER had reduced numbers of some T cell subsets per organ, but there were no differences when T cell subsets were expressed per g tissue. Both ZD and ER had elevated circulating corticosterone and reduced thymic pre-T cell numbers, but in the spleen ZD had fewer helper and cytolytic T cell numbers compared with ER and CTL. Although thymus and spleen Zn concentrations were unchanged, other variables in the periphery related to low Zn status might be contributing factors.

In summary, lymphoid cell numbers and lymphoid organ weights recover more rapidly from Zn deficiency and energy restriction in growing rats than body weight. Thus, the body produces more T lymphocytes and releases them into circulation for immune defence while nutritional recovery is in progress. Future studies need to assess the functional recovery of the immune system, including resistance to infection.

Acknowledgements

We thank the staff of the Animal Holding Facility and Amy Noto for their assistance. C. G. T. received funding from the Natural Sciences and Engineering Research Council of Canada.

References

Bises G, Mengheri E & Gaetani S (1987) T-lymphocyte subsets in zinc deficient and in protein malnourished rats. *Nutr Rep Int* **36**, 1371–1378.

Briefel RR, Bialostosky K, Kennedy-Stephenson J, McDowell MA, Ervin RB & Wright JD (2000) Zinc intake of the US population: Findings from the Third National Health and Nutrition Examination Survey, 1988–1994. *J Nutr* **130**, 1367S–1373S.

Cook-Mills JM & Fraker PJ (1993) Functional capacity of

residual lymphocytes from zinc deficient adult mice. *Br J Nutr* **69**, 835–848.
DePasquale-Jardieu P & Fraker PJ (1979) The role of corticosterone in the loss in immune function in the zinc-deficient A/J mouse. *J Nutr* **109**, 1847–1855.
Fraker PJ, DePasquale-Jardieu P, Zwickl CM & Luecke RW (1978) Regeneration of T-cell helper function in zinc-deficient adult mice. *Proc Natl Acad Sci USA* **75**, 5660–5664.
Fraker PJ, Haas SM & Luecke RW (1977) Effect of zinc deficiency on the immune response of the young adult A/J mouse. *J Nutr* **107**, 1889–1895.
Fraker PJ, King LE, Laakko T & Vollmer TL (2000) The dynamic link between the integrity of the immune system and zinc status. *J Nutr* **130**, 1399S–1406S.
Fraker PJ, Osati-Ahtiani F, Wagner MA & King LE (1995) Possible roles for glucocorticoids and apoptosis in the suppression of lymphopoiesis during zinc deficiency: A review. *J Am Coll Nutr* **14**, 11–17.
Fraker PJ, Zwickl CM & Luecke RW (1982) Delayed type hypersensitivity in zinc deficient adult mice: Impairment and restoration of responsivity to dinitrofluorobenzene. *J Nutr* **112**, 309–313.
Giugliano R & Millward DJ (1984) Growth and zinc homeostasis in the severely Zn-deficient rat. *Br J Nutr* **52**, 545–560.
Hosea HJ, Rector ES & Taylor CG (2003) Zinc-deficient rats have fewer recent thymic emigrant ($CD90^{+}$) T lymphocytes in spleen and blood. *J Nutr* **133**, 4239–4242.
Hughes FM & Cidlowski JA (1998) Glucocorticoid-induced thymocyte apoptosis: Protease-dependent activation of cell shrinkage and DNA degradation. *J Steroid Biochem Mol Biol* **65**, 207–217.
King LE, Osati-Ashtiani F & Fraker PJ (2002) Apoptosis plays a distinct role in the loss of precursor lymphocytes during zinc deficiency in mice. *J Nutr* **132**, 974–979.
Lepage LM, Giesbrecht JC & Taylor CG (1999) Expression of T lymphocyte $p56^{lck}$, a zinc-finger signal transduction protein, is elevated by dietary zinc deficiency and diet restriction in mice. *J Nutr* **129**, 620–627.
Moore JB, Blanchard RK, McCormack WT & Cousins RJ (2001) cDNA array analysis identifies thymic LCK as upregulated in moderate murine zinc deficiency before T-lymphocytes population changes. *J Nutr* **131**, 3189–3196.
Nobili F, Vignolini G, Figus E & Mengheri E (1997) Treatment of rats with dexamethasone or thyroxine reverses zinc deficiency-induced intestinal damage. *J Nutr* **127**, 1807–1813.
Sharon J (1998) *Basic Immunology*. Baltimore, MD: Williams & Wilkins.
Shipp K & Woodward BD (1998) A simple exsanguinations method that minimizes acute pre-anesthesia stress in the mouse: Evidence based on serum cortiocosterone concentrations. *Contemp Top Lab Anim Sci* **37**, 73–77.
Steel DM & Whitehead AS (1994) The major acute phase reactants: C-reactive protein, serum amyloid P component and serum amyloid A protein. *Immunol Today* **15**, 81–88.
Taylor CG, Potter AJ & Rabinovitch PS (1997) Splenocyte glutathione and CD3-mediated cell proliferation are reduced in mice fed a protein-deficient diet. *J Nutr* **127**, 44–50.
Woodward BD (1998) Protein, calories, and immune defences. *Nutr Rev* **56**, S84–S92.
Woodward BD (2003) Apoptotic loss of thymic lymphocytes in acute murine zinc deficiency (letter). *J Nutr* **133**, 814.