

# Reduction in serum leptin and IGF-1 but preserved T-lymphocyte numbers and activation after a ketogenic diet in rheumatoid arthritis patients

D.A. Fraser<sup>1,4</sup>, J. Thoen<sup>1</sup>, S. Bondhus<sup>1</sup>, M. Haugen<sup>1</sup>, J.E. Reseland<sup>2</sup>,  
O. Djøseland<sup>3</sup>, Ø. Førre<sup>1</sup>, J. Kjeldsen-Kragh<sup>4</sup>

---

<sup>1</sup>Centre for Rheumatic Diseases, The National Hospital; <sup>2</sup>Institute for Nutrition Research, University of Oslo; <sup>3</sup>Department of Endocrinology, The National Hospital; <sup>4</sup>Department of Immunology and Transfusion Medicine, Ullevaal University Hospital, Oslo, Norway.

---

## Abstract

### Objective

To assess the clinical, immunological and hormonal effects of carbohydrate restriction in rheumatoid arthritis (RA) patients via the provision of a ketogenic diet.

---

### Methods

Thirteen RA patients with active disease consumed a ketogenic diet for 7 days, providing the estimated requirements for energy and protein whilst restricting their carbohydrate intake to < 40 g/day. This was followed by a 2-week re-feeding period. Clinical and laboratory evaluations were carried out on days 0, 7 and 21. Changes in serum glucose,  $\beta$ -hydroxybutyrate ( $\beta$ -HB), leptin, insulin-like growth factor-1 (IGF-1) and cortisol were also measured at these time points. To study CD4+ and CD8+ lymphocyte responses, mitogen stimulated T-cell activation was assessed in heparinised whole blood via flow-cytometric analysis of CD69 expression.

---

### Results

After the 7-day ketogenic diet, there were significant increases in serum  $\beta$ -HB and cortisol, and significant decreases in body weight, the total lymphocyte count, serum leptin, IGF-1 and glucose. However, with the exception of morning stiffness, there were no significant changes in any of the clinical or laboratory measures of disease activity, or in early T-lymphocyte activation and the absolute numbers of CD4+ and CD8+ cells.

---

### Conclusion

In RA patients several of the metabolic and hormonal responses to a ketogenic diet, such as a fall in serum IGF-1 and leptin, resemble those which occur in response to acute starvation. However, the clinical and immunological changes which occur in response to acute starvation do not take place with a ketogenic diet and thus may be dependent upon energy and/or protein restriction.

---

### Key words

Rheumatoid arthritis, ketogenic diet, leptin, IGF-1, fasting, immune.

---

David A. Fraser, MSc; Jørn Thoen, MD, PhD; Sissel Bondhus, BSc; Margaretha Haugen, PhD; Janne E. Reseland, PhD; Ole Djøseland, PhD; Øystein Førre, MD, PhD; Jens Kjeldsen-Kragh, MD, PhD.

Please address correspondence and reprint requests to: David A. Fraser, Department of Immunology and Transfusion Medicine, Ullevaal University Hospital, N-0407, Oslo, Norway.

E-mail: DFRA@nycomed.com

© Copyright CLINICAL AND EXPERIMENTAL RHEUMATOLOGY 2000.

## Introduction

Beneficial effects of acute starvation upon both clinical and laboratory variables reflecting disease activity in rheumatoid arthritis (RA) have been documented (1-3). We have recently shown in RA patients that a 7-day fast, in addition to causing significant decreases in ESR, CRP, the tender joint count and morning stiffness, also decreases CD4+ lymphocyte activation and numbers whilst increasing IL-4 production from peripheral blood cells (4). Fasting has also been shown to decrease mitogen- and antigen-induced lymphocyte proliferative responses (5) and to suppress interleukin-2 production in healthy subjects (6).

Although it is well established that chronic sub-optimal protein and calorie intakes have a negative effect upon cell-mediated immune function (7, 8), the mechanisms underlying immunological changes associated with acute starvation in both healthy subjects and RA patients are unclear. The lack of any clinical or laboratory effects of an elemental diet does not support the idea that the avoidance of specific food items is responsible for the clinical effects of fasting in RA patients (9, 10).

Interestingly, it is known that carbohydrate restriction mediates many of the metabolic and hormonal responses to fasting (11, 12). Thus, the substitution of dietary carbohydrates with lipids results in similar decreases in blood glucose and insulin and increases in free fatty acids, ketone bodies and whole body lipolysis as would be observed during total fasting. Other hormonal adaptations to carbohydrate restriction may have important immunomodulatory effects. For example cortisol, which has well established anti-inflammatory effects, plays a counter-regulatory role in response to hypoglycaemia (13) and is increased by an isoenergetic carbohydrate restricted diet (14).

A reduction in blood glucose concentrations may also mediate acute changes in serum concentrations of leptin, the protein product of the *ob* gene (15-17). Recently it has been shown that the immunosuppressive effects of acute starvation in mice can be abolished by preventing the fasting-induced fall in plasma leptin

levels, suggesting that the reduced immune function associated with starvation may be leptin-mediated (18). We found that serum leptin concentrations were reduced by 67% after fasting in RA patients (4). As leptin enhances both naive CD4+ lymphocyte proliferative responses and Th1 cytokine production (18), a fall in serum leptin might explain both the increase in IL-4 production and the decrease in CD4+ T-cell activation production in mitogen stimulated peripheral blood cells we recently observed (4).

We speculated that the immunosuppressive and clinical effects of acute starvation in RA patients might be dependent upon hormonal adaptations to dietary carbohydrate restriction rather than upon energy and/or protein deprivation. We have therefore assessed the effects of a 7-day ketogenic diet (2000-2500 kcal/day primarily as lipids, 0.8 g protein/kg body weight/day, < 40 g carbohydrate/day) upon laboratory and clinical indices of disease activity in a group of 13 RA patients. We simultaneously assessed changes in T-cell number and function along with serum glucose, leptin, IGF-1, -hydroxybutyrate (-HB) and cortisol levels.

## Patients and methods

### Patients

Thirteen RA patients attending the outpatient department at the Centre for Rheumatic Diseases, The National Hospital, who satisfied the American College of Rheumatology criteria for RA (19) were recruited. All had active disease, as defined by the presence of 3 of the 4 following criteria; more than 3 swollen joints (28-joint score), more than 6 tender joints (28-joint score), duration of morning stiffness > 45 mins, erythrocyte sedimentation rate (ESR) > 28 mm in the 1st hour. All patients had radiological erosions of grade 3 or less, a body mass index (BMI) between 22 kg/m<sup>2</sup> and 28 kg/m<sup>2</sup> and had not previously participated in any dietary regime incorporating fasting, and were not on any self- or medically-prescribed diets which could influence the results. Patients with other serious medical conditions were not included in the trial. There were 12 women and 1 man, with a median age of 37 years (range 25-69 years) and a median dis-

ease duration of 4 years (range 0.2 - 20 years). Twelve patients were in functional class II and 1 was in functional class III (20). Eleven patients were rheumatoid factor positive (IgM). Four patients were taking second-line drugs, 4 were taking corticosteroids, 3 were taking cytostatic drugs, 13 were taking NSAIDs and 4 were taking painkillers. The corticosteroid dosage did not exceed 5 mg/day and this dose was stable for at least 4 weeks before study entry and during the trial. Patients using slow-acting anti-rheumatic or cytostatic drugs were on a stable dosage for at least 3 months prior to study entry. The dosage of NSAIDs was stable for 2 weeks prior to inclusion. Patients were instructed to maintain a stable dosage of their current drugs and no changes in drug type were permitted during the trial.

#### Study design

The study was approved by the regional scientific ethics committee. The 13 patients were contacted by telephone by a dietician to establish if there were any individual dislikes or preferences regarding the ketogenic diet. The diet consisted of selected vegetables, meat, fish, eggs, nuts, mayonnaise, olive oil, herbs and spices. It was prepared by a dietician and was varied as much as possible to improve palatability and thus compliance. The diet provided between 2000 and 2500 kcal (8.4- 10.5 MJ) per day depending upon body weight and provided 0.8 g protein/kg body weight per day and < 40 g carbohydrate/day. All patients were instructed to consume sufficient fluids in the form of water, tea, coffee (without milk) or other calorie-free drinks during the diet. After 7 days on the ketogenic diet, patients followed a lacto-vegetarian diet for a period of 2 weeks. The lacto-vegetarian diet was presented as an "experimental diet" and thus, to some degree, acted as a placebo for the ketogenic diet period. All patients were instructed to continue their normal everyday activities during both the ketogenic diet and the 2-week re-feeding period.

#### Clinical and laboratory assessment

All patients attended a baseline examination on the same day as the commencement of the ketogenic diet. They were

instructed to consume a normal breakfast before the evaluation. Two further clinical examinations were carried out on the last morning of the diet (day 7) and 2 weeks later on the completion of the re-feeding period (day 21). The following clinical variables were measured at baseline, on day 7 and on day 21 by the same examining physician: body weight, morning stiffness (min), the number of tender joints (28 joint score), and the number of swollen joints (28 joint score). At baseline, on day 7 and on day 21 peripheral blood samples were taken by venipuncture between 9:00 and 10:00 am. The ESR, CRP, platelet count and differential white blood cell count (WBC) were measured immediately at the Department of Clinical Chemistry, National Hospital, Oslo. Samples of heparinised blood (preservative-free sodium heparin 150 U.S.P. units) were also collected at all time points for analysis of lymphocyte activation. Serum samples were frozen at -70°C for subsequent analysis of cortisol, leptin, IGF-1, glucose and -HB.

#### Assays

T-cells were stimulated by a mixture of 2 monoclonal antibodies (mAbs) against CD2, clone L303.1, IgG2a, and CD2R, clone L304.1, and IgG1 (Becton Dickinson, San Jose, CA, USA) which when used together induce polyclonal T-cell activation. T-cells were stimulated or left resting by the addition or not of 5 µl CD2/CD2R (1 µg/µl) respectively to 250 µl aliquots of freshly isolated heparinised blood. These mixtures were then incubated for 4 hrs at 37°C in a humidified atmosphere containing 5% CO<sub>2</sub>.

After the incubation, aliquots of 50 µl from the samples with activated cells and aliquots of 50 µl from the samples with resting cells were incubated for 30 min with the following fluorochrome conjugated mAbs: anti-CD69, clone L78, IgG1, PE conjugated (Becton Dickinson) + anti-CD4, clone RPA-T4, IgG1, FITC conjugated (Serotec, Oxford, England) + anti-CD8, clone SK1, IgG1, PerCP conjugated (Becton Dickinson). Mouse IgG1, clone X40, conjugated either with PE, FITC and PerCP (all from Becton Dickinson) were used as control antibodies. After 30 min of incubation at room

temperature the erythrocytes were lysed by a Multi-Q-Prep (Coulter, Hialeah, FL, USA). The samples were then analysed by a FACScan or FACSCalibur (Becton Dickinson) flow cytometer.

The lymphocytes were identified on the forward light scatter versus side light scatter plot. The percentage of CD69-positive cells was obtained by subtracting the percentage of positive events in the isotype control sample from the percentage of positive events in the anti-CD69 antibody-stained sample. Lymphocyte subset numbers were quantified using FlowCount fluorospheres (Coulter) by adding a known amount of fluorospheres to each tube immediately prior to sample analysis on the flow cytometer. The fluorospheres were gated on the FL1 and FL2 channels and their numbers obtained, and the numbers of CD4+ and CD8+ lymphocytes were similarly gated and counted on the FL1 and FL3 channels respectively. The number of fluorospheres sampled by the flow cytometer as a proportion of the total amount added was then used to calculate the absolute number of CD4+ and CD8+ lymphocytes.

Serum cortisol, leptin and IGF-1 were measured using commercially available radioimmunoassay kits (Orion Diagnostica, Espoo, Finland; Linco Research, St. Charles, USA; and Nichols Institute Diagnostics, CA, USA respectively). Serum glucose and -HB were measured enzymatically (Boehringer Mannheim, Mannheim, Germany; and Sigma, St. Louis, MO, USA). Normal reference values for apparently healthy subjects (supplied by manufacturer) for cortisol are 167 - 682 nmol/l (8-10 am) and for fasting glucose are 3.9 - 5.8 mmol/l.

#### Statistical analysis

Within group differences were tested by the Wilcoxon signed-rank test. Two-sided p values of < 0.05 were considered as significant. The analyses was carried out using STATVIEW 4.1 (Abacus Concepts, Berkeley, CA).

## Results

#### Clinical response

As is shown in Table I, there were no significant decreases in any of the objective variables of disease activity at any

time point. With regard to the subjective variables, there was a significant decrease in morning stiffness at the conclusion of the ketogenic diet.

*Metabolic and hormonal response*

We found a significant decrease in body weight after the ketogenic diet as compared to baseline (Table II). There was a significant increase in serum cortisol levels after the ketogenic diet as compared with baseline. The median -HB concentration on day 7 of the ketogenic diet was significantly increased as compared to baseline, whereas serum glucose concentrations were significantly decreased. At the conclusion of the ketogenic diet, both median serum leptin and IGF-1 levels had significantly decreased by almost 50%. After re-feeding, only body weight remained significantly reduced as compared to baseline, although this difference was negligible. No significant differences were observed for any other variables at day 21 compared to baseline.

*Total and differential WBC and CD4+ and CD8+ counts*

There was a small but significant decrease in the absolute lymphocyte count after the ketogenic diet, but no changes in the total WBC or CD4+ and CD8+ counts or in the number of CD4+ and CD8+ lymphocytes as a percentage of total lymphocytes at any time point (Table III).

*Mitogen induced activation of CD4+ and CD8+ cells*

To assess whether the ketogenic diet had an inhibitory effect upon lymphocyte activation, we studied the expression of the early activation marker, CD69, on CD4+ and CD8+ lymphocytes after mitogenic stimulation in whole blood. We used whole blood in preference to traditional methods involving the isolation of mononuclear cells as we considered that changes which might occur in the plasma concentrations of hormones and fuels in response to the ketogenic diet could have important effects upon T-cell function. However, despite the changes we observed in the concentrations of IGF-1, leptin and cortisol, we could not detect any significant changes in CD69 expres-

sion on either CD4+ or CD8+ cells at any time point (Table III).

**Discussion**

In this study we have investigated whether a ketogenic diet would modulate immune function and improve disease activity in RA patients, as we recently

observed in response to fasting (4). Our initial hypothesis was that metabolic and hormonal adaptations to carbohydrate restriction, as opposed to energy restriction, could be responsible for the observed effects of fasting. If this were true, the provision of a ketogenic diet should reproduce the beneficial effects of fast-

**Table I.** Effects of ketogenic diet upon clinical and laboratory variables.<sup>1</sup>

	Baseline	Day 7 of ketogenic diet	After 2 weeks of re-feeding
Tender joints (28-joint score) NS	12 (6 - 16)	8 (5 - 14)	10 (6 - 16)
Swollen joints (28-joint score) NS	5 (4 - 12)	7 (4 - 13)	10 (4 - 14)
Morning stiffness (mins)	120 (60 - 120)	60 (30 - 120) <sup>2</sup>	90 (45 - 120)
ESR (mm/h) NS	28 (20 - 48)	28 (16 - 40)	30 (18 - 62)
CRP (mg/l) NS	13 (5 - 61)	19 (9 - 56)	12 (5 - 44)

<sup>1</sup>Median (95% confidence intervals); n = 13. Significantly different from baseline (Wilcoxon signed rank) <sup>2</sup>p < 0.05. NS = no significant changes at any timepoint.

**Table II.** Effects of a 7-day ketogenic diet upon body weight, serum cortisol, glucose, -HB, leptin and IGF-1.<sup>1</sup>

	Baseline	Day 7 of ketogenic diet	After 2 weeks of re-feeding
Body weight (kg)	65.9(60-80.4)	63 (58.2-77.6) <sup>3</sup>	64.9 (59-79.2) <sup>3</sup>
Cortisol (nmol/l)	325(272-475)	371(320-577) <sup>2</sup>	319 (269-439)
Glucose (mmol/l)	5.0 (4.5-5.4)	3.8 (2.9-4.4) <sup>3</sup>	4.8 (4.5-5.2)
-HB (mmol/l)	< 0.1	2.6 (1.6-3.8) <sup>3</sup>	< 0.1
Leptin (ng/ml)	12.5 (6.4-20)	6.6 (4.3-8.2) <sup>3</sup>	12.8 (8.2-20)
IGF-1 (nmol/l)	16.6 (12-21.1)	9.0 (1.0-12.1) <sup>3</sup>	15.7 (11.9-22.2)

<sup>1</sup>Median (95% confidence intervals); n = 13. Significantly different from baseline (Wilcoxon signed rank) <sup>2</sup>p < 0.02, <sup>3</sup>p < 0.005.

**Table III.** Effects of ketogenic diet upon total lymphocyte, CD4+ and CD8+ counts and percentage of CD4+ and CD8+ cells expressing CD69 in mitogen stimulated whole blood.<sup>1</sup>

	Baseline	Day 7 of ketogenic diet	After 2 weeks of re-feeding
Leukocyte count (10 <sup>9</sup> /l) NS	7.8 (5-10.4)	7.0 (5.2-12.2)	6.9 (5.5-11.3)
Lymphocyte count (10 <sup>9</sup> /l)	1.58 (1.4-2.03)	1.49 (1.10-1.97) <sup>2</sup>	1.54(1.24-2.52)
CD4+ (cells / ml) NS	690 (480-900)	720 (400-900)	700 (470-900)
CD8+ (cells / ml) NS	250 (190-340)	270 (140-360)	250 (160-300)
CD4+ (% of total lymphocytes) NS	41 (30-56)	42 (31-51)	42 (26-53)
CD8+ (% of total lymphocytes) NS	15 (12-19)	15 (11-20)	15 (9-17)
CD4+CD69+ (% of CD4+ cells) NS	72 (61-81)	69 (59-84)	74 (63-81)
CD8+CD69+ (% of CD8+ cells) NS	71 (58-86)	69 (61-82)	73 (64-83)

<sup>1</sup>Median (95% confidence intervals); n = 13. Significantly different from baseline (Wilcoxon signed rank) <sup>2</sup>p < 0.03. NS = no significant changes at any timepoint.

ing upon disease activity without compromising the patient's nutritional status. However, as we did not observe any changes in either the laboratory or clinical variables, with the exception of morning stiffness, this hypothesis does not appear to hold. We must, however, also take into account that we compared the effects of a ketogenic diet with results from our earlier investigation into the effects of fasting, i.e. the two groups were not directly compared. Although there were no significant differences between the patients in the fasting study and the present study with regard to age or disease duration, additional confounding factors may have had an important bearing on the outcomes of the respective regimes. We must also take into account the fact that caution should be observed when drawing negative conclusions based on a small number of patients.

To achieve our objectives and to encourage patient compliance, it was necessary to design a diet which was palatable yet fulfilled the required criteria regarding carbohydrate content. At the conclusion of the ketogenic diet, all patients stated that the diet was palatable and that they were able to complete the protocol as instructed. The use of a ketogenic diet is well documented in the treatment of childhood epilepsy and appears to have no apparent side effects when followed over a period of several years (21). The significant increases in  $\gamma$ -HB and the decrease in glucose concentrations to levels similar to those observed in response to fasting levels provide some indirect evidence that the patients did consume the provided diet as claimed (22).

As the patients were allowed to live at home for the duration of the study, it is possible that the increase in  $\gamma$ -HB levels resulted from the patients simply fasting. However, we observed that the median body weight was only 1 kg less than baseline at 2 weeks after the ketogenic diet as compared to the 3.5 kg weight loss which was still present 2 weeks after sub-total fasting (4). Given that the re-feeding diets were similar in both studies, it would appear that a much greater calorie deficit occurred during the fasting period compared to the ketogenic diet

period. The significant weight loss which we observed at day 7 of the ketogenic diet was most likely associated with a temporary negative fluid balance secondary to natriuresis. Sodium excretion during carbohydrate restriction has been shown to mimic that which occurs in response to fasting (23).

Although we found a significant increase in serum cortisol concentrations after the ketogenic diet, the increase was apparently not sufficient to reduce disease activity or influence the T-lymphocyte number or functioning. The 15% increase in serum cortisol we observed is comparable with the 22% increase observed by Phinney *et al.* in healthy subjects after a ketogenic diet (24). We did not determine 24-hour cortisol concentrations, and therefore cannot estimate the true magnitude of the changes in cortisol production induced by the ketogenic diet. However, our results do not support the idea that a counter-regulatory increase in serum cortisol concentrations in response to carbohydrate restriction has immunomodulatory or clinically relevant effects in RA patients. Nevertheless, it is quite possible that other factors associated with the stress of acute energy deprivation stimulate additional cortisol secretion in fasting RA patients.

A fall in serum leptin has been implicated as a pivotal factor in starvation-induced immunosuppression (18). Increasing concentrations of leptin, levels of which are directly correlated with fat mass (25), enhance T-cell proliferation and promote a Th1 response *in vitro* (18). An increased Th1 response may be detrimental in RA (26). These findings are also of interest in relation to the finding that a BMI > 30 kg/m<sup>2</sup> is associated with an adjusted odds ratio of 3.74 for developing RA (27). Our finding that the ketogenic diet induced falls in leptin levels approaching those observed in response to sub-total fasting in the absence of changes in T-cell number or activation does not support the idea that leptin plays a pivotal role in the fasting-induced suppression of CD4<sup>+</sup> cell activation and numbers we previously observed (4). The unchanged laboratory and clinical variables in response to the ketogenic diet would also suggest that clinically

relevant immunosuppression did not occur.

The findings of Lord *et al.* that leptin plays a pivotal role in fasting-induced immunosuppression were carried out in mice, where a 2-day fast reduced body weight by as much as 20-30% (18). Their findings may thus be more relevant to chronic malnutrition in humans, where a greater loss of body mass occurs. In our study, the ketogenic diet reduced leptin levels by 47% as compared to a 67% reduction after sub-total fasting (4). We therefore cannot rule out the possibility that the greater decrease in serum leptin after sub-total fasting is of relevance to the observed immunosuppression.

The 46% decrease in serum IGF-1 levels observed at the conclusion of the ketogenic diet approached the 55% reduction we previously noted after 7 days of acute starvation (4). Serum IGF-1 is a marker of nutritional status and has been shown to correlate with nitrogen balance (28). The fall we observed in IGF-1, despite the provision of adequate dietary protein and calories, may be due to the fact that a ketogenic diet induces a negative nitrogen balance for a period of around one week before adaptation occurs (24).

The marked reduction we observed in serum IGF-1 could have immunomodulatory effects, as IGF-1 is known to enhance T-cell proliferation (29). It has previously been suggested that the T-cell suppression observed during fasting may be secondary to falls in serum IGF-1 (6). However, as we did not observe any immunological effects of the ketogenic diet, we do not suspect the acute falls in IGF-1 of the magnitude we observed induce decreases in T-cell numbers and function.

In summary, using a ketogenic diet we have found metabolic and hormonal adaptations to carbohydrate restriction do not induce clinical improvements or immunomodulatory changes in RA patients. The lack of any clinical or laboratory improvements in response to the ketogenic diet suggest that the reductions we observed in serum IGF-1 and leptin do not appear to have clinically relevant effects. Although we cannot yet identify specifically which factors mediate either

the immunosuppression or the clinical effects of fasting in RA patients, additional factors associated with acute energy and/or protein deficiency may be important.

### Acknowledgements

We wish to thank Dr. Anne-Marit Selvaag at the Centre for Rheumatic Diseases for her clinical assistance, and the staff personnel at the Centre for Rheumatic Diseases, the Department of Clinical Chemistry and the Department of Endocrinology, The National Hospital for their technical assistance.

### References

- SKÖLDSTAM L, LARSSON L, LINDSTRÖM FD: Effects of fasting and lactovegetarian diet on rheumatoid arthritis. *Scand J Rheumatol* 1979; 8: 249-55.
- HAFSTRÖM I, RINGERTZ B, GYLLENHAMMER H, PALMBLAD J, HARMS-RINGDAHL M: Effects of fasting in disease activity, neutrophil function, fatty acid composition, and leukotriene biosynthesis in patients with rheumatoid arthritis. *Arthritis Rheum* 1988; 31: 585-92.
- KJELDSEN-KRAGH J, HAUGEN M, BORCHGREVINK CF *et al.*: Controlled trial of fasting and one-year vegetarian diet in rheumatoid arthritis. *Lancet* 1991; 338: 899-902.
- FRASER D, THOEN J, RESELAND J, FØRRE Ø, KJELDSEN-KRAGH J: Decreased CD4+ lymphocyte activation and increased IL-4 production in peripheral blood of rheumatoid arthritis patients after acute starvation. *Clin Rheumatol* 1999; 18: 394-401.
- HOLM G, PALMBLAD J: Acute energy deprivation in man: effect on cell-mediated immunological reactions. *Clin Exp Immunol* 1976; 25: 207-11.
- SAVENDAHL L, UNDERWOOD L: Decreased interleukin-2 production from cultured peripheral blood mononuclear cells in human acute starvation. *J Clin Endocrinol Metab* 1997; 82: 1177-80.
- WOODWARD B: Protein, calories and immune defences. *Nutr Rev* 1998; 56: S84-S92.
- KRAMER T, MOORE R, SHIPPEE R *et al.*: Effects of food restriction in military training on T-lymphocyte responses. *Int J Sports Med* 1997; 18: S84-S90.
- VAN DE LAAR MAFI, VAN DER KORST JK: Food intolerance in rheumatoid arthritis. I. A double blind, controlled trial of the clinical effects of elimination of milk allergens and azo dyes. *Ann Rheum Dis* 1992; 51: 298-302.
- HAUGEN M, KJELDSEN-KRAGH J, FØRRE Ø: A pilot study of the effect of an elemental diet in the management of rheumatoid arthritis. *Clin Exp Rheumatol* 1994; 12: 275-9.
- KLEIN S, WOLFE R: Carbohydrate restriction regulates the adaptive response to fasting. *Am J Physiol* 1992; 262: E631-6.
- FERY F, BOURDOUX P, CHRISTOPHE J, BALLASSE E: Hormonal and metabolic changes induced by an isocaloric isoproteic ketogenic diet in healthy subjects. *Diabetes Metab* 1982; 8: 299-305.
- DE FEO P, PERRIELLO G, TORLONE E *et al.*: Contribution of cortisol to glucose counter-regulation in humans. *Am J Physiol* 1989; 257: E35-E42.
- LANGFORT J, PILIS W, ZARZECZNY R, NAZAR K, KACIUBA-USCILKO H: Effect of low-carbohydrate-ketogenic diet on metabolic and hormonal responses to graded exercise in men. *J Physiol Pharmacol* 1996; 47: 361-71.
- BODEN G, CHEN X, MOZZOLI M, RYAN I: Effect of fasting on serum leptin in normal human subjects. *J Clin Endocrinol Metab* 1996; 81: 3419-23.
- GRINSPOON S, ASKARI H, LANDT M *et al.*: Effects of fasting and glucose infusion on basal and overnight leptin concentrations in normal-weight women. *Am J Clin Nutr* 1997; 66: 1352-6.
- TUOMINEN J, EBELING P, LAQUIER F, HEIMAN M, STEPHENS T, KOIVISTO V: Serum leptin concentration and fuel homeostasis in healthy man. *Eur J Clin Invest* 1997; 3: 206-11.
- LORD G, MATARESE G, HOWARD J, BAKER R, BLOOM S, LECHLER R: Leptin modulates the T-cell immune response and reverses starvation-induced immunosuppression. *Nature* 1998; 394: 897-901.
- ARNETT FA, EDWORTHY SM, BLOCH DA *et al.*: The American Rheumatism Association 1987 revised criteria for the classification of rheumatoid arthritis. *Arthritis Rheum* 1988; 31: 315-24.
- STEINBROCKER O, TRAEGER CH, BATTERMAN RC: Therapeutic criteria in rheumatoid arthritis. *JAMA* 1949; 140: 659-62.
- KINSMAN SL, VINING EPG, QUASKEY SA, MELLITS D, FREEMAN JM: Efficacy of the ketogenic diet for intractable seizure disorders: Review of 58 cases. *Epilepsia* 1992; 6: 1132-6.
- CARLSON MG, SNEAD WL, CAMPBELL PJ: Fuel and energy metabolism in fasting humans. *Am J Clin Nutr* 1994; 60: 29-36.
- BOULTER P, HOFFMAN R, ARKY R: Pattern of sodium excretion accompanying starvation. *Metab Clin Exp* 1973; 22: 675-83.
- PHINNEY S, BISTRAN B, WOLFE R, BLACKBURN G: The human metabolic response to chronic ketosis without caloric restriction: Physical and biochemical adaptation. *Metabolism* 1983; 32: 757-68.
- CONSIDINE R: Weight regulation, leptin and growth hormone. *Horm Res* 1997; 48 (Suppl. 5): 116-21.
- MIOSSEC P, CHOMARAT P, DECHANET J: Bypassing the antigen to control rheumatoid arthritis. *Immunol Today* 1996; 17: 170-3.
- SYMMONS D, BANKHEAD C, HARRISON B *et al.*: Blood transfusion, smoking and obesity as risk factors for the development of rheumatoid arthritis: Results from a primary care-based incident case-control study in Norfolk, England. *Arthritis Rheum* 1997; 40: 1955-61.
- ISLEY WL, UNDERWOOD LE, CLEMMONS DR: Dietary components that regulate serum somatomedin-C concentrations in humans. *J Clin Invest* 1983; 71: 175-182.
- JOHNSON E, JONES L, KOZAC R: Expression and function of insulin-like growth factor receptors on anti-CD3-activated human T lymphocytes. *J Immunol* 1992; 148: 63-71.