

Serum levels of interleukin-6 and dehydroepiandrosterone sulphate in response to either fasting or a ketogenic diet in rheumatoid arthritis patients

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Abstract

Objective

To investigate the effects of either a 7-day fast or a 7-day ketogenic diet upon serum interleukin-6 (IL-6) and dehydroepiandrosterone sulphate (DHEAS) in RA patients.

Methods

We measured serum concentrations of DHEAS and IL-6 in 23 RA patients with active disease, 10 of whom followed a 7-day sub-total fast and 13 of whom consumed a ketogenic diet (isoenergetic, carbohydrate < 40 g/day) for 7 days. Clinical and laboratory variables were measured at baseline, on day 7 and after re-feeding on day 21. Correlation analyses were used to assess the associations between serum IL-6, DHEAS and disease activity variables at each timepoint.

Results

Fasting, but not the ketogenic diet, decreased serum IL-6 concentrations by 37% ($p < 0.03$) and improved disease activity at day 7. Both fasting and the ketogenic diet increased serum DHEAS levels by 34% as compared with baseline (both $p < 0.006$). Levels of IL-6, but not DHEAS, correlated with several disease activity variables.

Conclusion

Both fasting and a ketogenic diet significantly increased serum DHEAS concentrations in RA patients. Only fasting significantly decreased serum IL-6 levels and improved disease activity. As the increases in serum DHEAS were similar in response to both fasting and a ketogenic diet, it is unlikely that the fall in serum IL-6 or clinical improvements after fasting were directly related to increases in serum DHEAS. The fasting-induced fall in serum IL-6 may underlie the fall in CRP and ESR observed in RA patients in response to a 7-day fast.

Key words

Rheumatoid arthritis, fasting, ketogenic, dehydroepiandrosterone sulphate, interleukin-6.

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Introduction

Lowered plasma levels of the adrenal androgens (AA) dehydroepiandrosterone (DHEA) and dehydroepiandrosterone sulphate (DHEAS) have recently been found in women with premenopausal onset RA, both under basal conditions and in response to stimulation with adrenocorticotrophic hormone (ACTH), as compared with matched controls (1). Clinical and experimental evidence has suggested that AA may be involved in the pathophysiology of rheumatoid arthritis (RA) (2). DHEAS decreases the expression and activity of the interleukin (IL)-6 gene promoter (3), and IL-6 has been found to be inversely correlated with serum DHEAS levels both in RA patients and in healthy individuals (1, 4). Since IL-6 appears to play an important role in the development and manifestations of RA (5, 6), low concentrations of DHEAS may thus contribute to the pro-inflammatory cytokine mediated pathophysiology of RA.

Fasting is a reproducible way of transiently improving disease activity in RA patients (7-10). We have recently used fasting as a model for studying the hormonal and immunological changes which are associated with improvements in disease activity (11). As increases in serum DHEAS have previously been reported during fasting in humans (12, 13), and given the reported association between IL-6 and DHEAS, we decided to investigate how fasting affected serum IL-6 and DHEAS in 10 RA patients who underwent a 7-day fast followed by a 2-week re-feeding period.

We also measured serum IL-6 and DHEAS in 13 RA patients who followed a 7-day ketogenic diet (isoenergetic, carbohydrate restricted to < 40 g/day) followed by a 2-week re-feeding period (14). The ketogenic diet provides a useful comparison to fasting as a ketogenic diet induces many of the adaptive metabolic and hormonal responses which occur in response to fasting (15), yet has little effect upon disease activity or immune function in RA patients (14).

Patients and methods

Patients

For both the fasting and the ketogenic diet study, RA patients attending the out-

patient department at the Centre for Rheumatic Diseases, The National Hospital, who satisfied the American College of Rheumatology criteria for RA (16) and were willing to participate in the study were recruited. For both the fasting and ketogenic diet studies, selection was based both on fulfilment of the inclusion criteria and a willingness to participate in the studies and was carried out by the same physician (J.T). For practical purposes, the first 10 patients recruited were allocated to the fasting protocol, whilst the next 13 patients were allocated to the ketogenic diet group. When the fasting study was completed, recruitment for the ketogenic diet study commenced immediately.

In the fasting study there were 9 females and 1 male, with a mean age of 49 years (range 31-65 years) and a mean disease duration of 4.2 years (range 0.2 - 12 years). Nine patients were in functional class II with one in functional class III (17). Eight patients were rheumatoid factor positive (IgM). Five patients were taking second-line drugs, 1 was taking prednisolone, 8 were taking NSAIDs and 3 were taking painkillers.

For the ketogenic diet study there were 12 females and 1 male, with a mean age of 44 years (range 25 - 69 years) and a mean disease duration of 4.8 years (range 0.2 - 20 years). Twelve patients were in functional class II with one in functional class III (17). Eleven patients were rheumatoid factor positive (IgM). Four patients were taking second-line drugs, 4 were taking prednisolone, 13 were taking NSAIDs and 4 were taking painkillers.

In both studies the prednisolone dosage did not exceed 7.5 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 drugs were on a stable dosage for at least 3 months prior to study entry. The dosage of NSAIDs was stable for at least 2 weeks prior to inclusion. Patients were instructed to maintain their current medication during the study. Transient changes in the dosage of painkillers were permitted.

Study design

In the fasting study, all patients attended

a health farm where they immediately underwent a 7-day supervised sub-total fast. A limited amount of vegetable juices were permitted (< 50 g carbohydrate/day, total energy < 865 kJ (205 kcal)/day) (11). In the ketogenic diet study, all patients followed a ketogenic diet for 7 days consisting of selected vegetables, meat, fish, eggs, nuts, mayonnaise, olive oil, herbs and spices providing between 2000 and 2500 kcal (8.4-10.5 MJ) per day depending upon body weight, including 0.8 g protein/kg body weight per day and < 40 g carbohydrate/day (14).

Patients in both groups were encouraged to rest during the first 7 days of the study and to avoid any strenuous activities. No formal work was undertaken in the first 7 days of either protocol. Patients could return to work after the commencement of the lacto-vegetarian diet period.

After 7 days of either fasting or ketogenic diet, all patients followed a 2-week re-feeding period in which they consumed a lacto-vegetarian diet. The lacto-vegetarian diet was presented as an "experimental diet" and thus, to a degree, served as a placebo for the fasting and ketogenic diet periods.

Measurement of clinical, laboratory and hormonal variables

All patients attended a morning baseline examination either on the first day of the fast or ketogenic diet, or on the preceding day. They were instructed to consume a normal breakfast before the evaluation. Two further clinical examinations were carried out on the last morning of the fast or ketogenic diet (day 7) and 2 weeks later on the completion of the re-feeding period (day 21). The examinations were carried out by the same examining physician for each patient, with the exception of two instances where the examining physician at baseline and day 7 was not available at day 21.

Peripheral blood samples were taken by venipuncture between 10:30 and 11:30 am in the fasting study, and between 9:00 and 10:00 am in the ketogenic diet study. Standard laboratory techniques were used to measure ESR and CRP. Serum samples were frozen at -70°C for subsequent analysis of IL-6, DHEAS, cortisol and the ketone body, beta-hydroxy-

butyrate (-HB). -HB was measured to assess compliance with the fasting and ketogenic diet protocols. IL-6, DHEAS and -HB were analysed simultaneously for both study groups, whereas cortisol analysis was carried out first for the patients in the fasting study, and subsequently for the patients in the ketogenic diet study. Serum IL-6 was measured using a commercially available ELISA kit (R&D Systems, Abingdon, UK) with an assay sensitivity of 0.70 pg/ml and an inter-assay variation and intra-assay variation of 6.4% and 4.2% respectively. Serum DHEAS and cortisol were measured using commercially available radioimmunoassay kits (Diagnostic Product Corporation, USA, Nichols Inst. Diagnostics, USA and Orion Diagnostica, Espoo, Finland, respectively). Inter-assay variations were 8.3% and 2.8% while intra-assay variations were 4.7% and 7.0% for DHEAS and cortisol respectively. Serum -HB was measured enzymatically (Sigma, St. Louis, MO, USA).

Statistical analysis

Within group differences were tested by the Wilcoxon signed-rank test. Correlations between variables were calculated by the Kendall correlation analysis. Between group differences were tested by the Mann-Whitney U 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

As shown in Table I, we found a significant decrease (37%) in the concentration of serum IL-6 at day 7 of the fasting study. IL-6 values were also significantly lower at day 7 as compared to after re-feeding. However in the ketogenic diet study there were no significant changes in the concentration of serum IL-6 at any timepoint. We also found significant improvements in ESR, CRP and tender joint count at day 7 of fasting, whereas no changes were noted in disease activ-

Table I. Effects of a 7-day sub-total fast and a 7-day ketogenic diet upon serum IL-6, DHEAS, disease activity and metabolic variables¹.

	Baseline	Day 7	After 2 weeks re-feeding
Fasting			
IL-6 (pg/ml)	35.5 (23-71)	22.5 (18-28) ²	31.0 (19-48)
DHEAS (mmol/l)	3.28 (1.08-4.81)	4.40 (1.17-6.66) ³	3.58 (1.17-4.36)
ESR (mm/h)	33 (22-54)	21 (10-48) ⁵	29 (15-52)
CRP (mg/l)	25 (13-47)	13 (7-33) ⁴	21 (10-53)
Tender joints (28 score)	14 (8-21)	10 (2-17) ²	15 (5-18)
Cortisol (nmol/l) NS	317 (245-380)	280 (189-389)	274 (220-296)
-HB (mmol/l)	< 0.1	2.1 (0.8-4.3) ³	< 0.1
Body weight (kg)	70.5 (69.2-79.4)	67.5 (65.0-76.9) ³	67(66.1-76.5)
Ketogenic diet			
IL-6 (pg/ml) NS	17.0 (11-52)	21.0 (13-34)	22.0 (9-54)
DHEAS (mmol/l)	2.42 (0.95-4.16)	3.23 (1.20-5.47) ³	2.53 (1.12-4.50)
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)
Tender joints (28 score) NS	12 (6-16)	8 (5-14)	10 (6-16)
Cortisol (nmol/l)	325 (272-475)	371(320-577) ²	319 (269-439)
-HB (mmol/l)	< 0.1	2.6 (1.6-3.8) ³	< 0.1
Body weight (kg)	65.9 (60-80.4)	63 (58.2-77.6) ³	64.9 (59-79.2)

¹Median (95% confidence intervals); n = 13 for ketogenic diet study and n = 10 for fasting study. Significantly different from both baseline and after 2-weeks re-feeding (Wilcoxon signed rank)

²*p* < 0.05, ³*p* < 0.01.

Significantly different from baseline (Wilcoxon signed rank) ⁴*p* < 0.05, ⁵*p* < 0.01.

NS = no significant changes at any time-point.

ity variables during the ketogenic diet study at any time-point.

Serum DHEAS concentrations were significantly increased (by 34%) at day 7 as compared to baseline after both acute starvation and the ketogenic diet. Values at day 7 were also significantly higher as compared with day 21 (after re-feeding) after both interventions (Table I). Cortisol was significantly increased at day 7 of the ketogenic diet, but not in the fasting study (Table I). There were significant increases in serum γ -HB at day 7 of both interventions providing indirect evidence that the patients complied with the respective protocols as instructed.

We performed correlation analyses between serum IL-6 values and disease activity variables at individual time-points. Statistically significant correlations were found between IL-6 and tender joint count at baseline ($r = 0.57$, $p < 0.03$) and between IL-6 and CRP at day 7 ($r = 0.55$, $p < 0.03$) in the fasting patients and between IL-6 and ESR ($r = 0.47$, $p < 0.05$), CRP ($r = 0.47$, $p < 0.05$) and tender joint count ($r = 0.55$, $p < 0.02$) at day 21 in the ketogenic diet patients. As IL-6 is a potent stimulus of the hypothalamic-pituitary adrenal axis, we performed correlation analysis between IL-6 and cortisol. We found that IL-6 concentrations correlated with cortisol at baseline ($r = 0.63$, $p < 0.02$) and day 21 in the fasting patients ($r = 0.54$, $p < 0.03$). We then assessed whether there was any association between serum DHEAS concentrations and disease activity variables at individual time points. The only statistically significant correlation in the fasting study was between DHEAS and ESR at day 21 ($r = 0.49$, $p < 0.05$). We did not find any significant correlations between increases in serum DHEAS between baseline and day 7 (calculated as ratio of baseline values to values at day 7) and changes in serum IL-6 concentrations between baseline and day 7 (calculated as for DHEAS).

Discussion

The fall in serum IL-6 we observed in the fasting patients is likely to represent a reduction in IL-6 production in the synovium. Synovium derived IL-6 is the principal regulator of human acute-phase

protein synthesis in RA patients (18, 19), which explains the correlations we found between IL-6 concentrations and disease activity variables in both groups. Thus the reduction in IL-6 is likely, at least partly, to be responsible for the decrease in CRP. As ESR is reflective of serum concentrations of the acute-phase protein, fibrinogen, the reduction in IL-6 may also be responsible for the decrease we observed in ESR.

The ability of fasting to reduce serum IL-6 is a property shared by a number of therapies used in the treatment of RA, including tumour necrosis factor- α (TNF- α) blocking agents (20), and suggests that fasting may interfere with the cytokine network in the synovium. A number of studies have shown that IL-6 production in the synovium is largely mediated by macrophage-derived IL-1 and TNF- α . The production of IL-1 and TNF- α from macrophages is in turn thought to be T-cell dependent and the early T-cell activation marker, CD69, plays an important role in the contact-dependent T-cell activation of macrophages and subsequent IL-1 and TNF- α production (21, 22). With regard to the potential mechanisms which could explain the decrease we found in serum IL-6 after fasting, it is therefore interesting to note that we previously found significant decreases in CD69 expression on mitogen-stimulated CD4 $^{+}$ lymphocytes in whole blood at day 7 of fasting (11), but not after a ketogenic diet (14). However, although it would appear that short-term fasting has beneficial effects upon disease activity in RA, the impossibility of prolonged fasting prevents its use as a therapeutic option in the treatment of RA. Furthermore, the rapidity with which symptoms return upon the cessation of fasting does not support the possibility that intermittent fasting would be beneficial (11).

The 34% increase in serum DHEAS we observed both after fasting and a ketogenic diet corresponds to the increases observed by Komaki *et al.* (12), and suggests that carbohydrate restriction, as opposed to energy restriction, mediates this increase. Although ACTH is the dominant hormone controlling adrenal gland production of both cortisol and DHEAS, there are a number of other fac-

tors or hormones distinct from ACTH which regulate the secretion of human adrenal androgens (23-25). This may explain why we found no increase in serum cortisol concentrations at day 7 of fasting. Alternatively, the increase in serum DHEAS levels we observed may not reflect an increased production of DHEAS, but a decreased renal clearance. This has been reported in obese individuals in response to fasting (26) and may result from increased binding of DHEAS to albumin (13). There are therefore a number of mechanisms by which either fasting or the ketogenic diet could have increased serum DHEAS.

We found that cortisol was significantly increased in the ketogenic group, but not after fasting. This is somewhat unexpected given the positive clinical response and fall in IL-6 in response to fasting, but not after a ketogenic diet. This may be explained by the fact that cortisol, in contrast to DHEAS, has a pronounced circadian rhythm. Furthermore, in healthy individuals, fasting modifies the diurnal secretory pattern of cortisol by such that peak concentrations occur in the early afternoon as opposed to morning (27). It is therefore difficult to draw definitive conclusions regarding 24 hr cortisol concentrations from morning samples.

Cutolo *et al.* (1) found that IL-6 levels were rapidly reduced by adrenal gland steroids in response to ACTH stimulation. We, however, noted that despite similar increases in serum DHEAS in response to fasting and a ketogenic diet, a significant decrease in serum IL-6 was only found in the fasting patients. It would therefore appear that the increase in cortisol which we observed at day 7 of the ketogenic diet was not sufficient to decrease serum IL-6 levels. These findings also suggest that the increase in serum DHEAS levels may not have been responsible for the reduction in IL-6 seen in the fasting patients. A similar argument could be applied to the clinical response, which was favourable only in the fasting patients despite comparable increases in DHEAS in both groups. Although we cannot predict whether long-term increases in DHEAS would affect disease activity, acute increases of the magnitude we observed do not appear

to be of clinical relevance. These findings would tend to favour the hypothesis, as discussed by Masi *et al.* that lowered DHEAS levels are a marker of general hypothalamic-pituitary-adrenocortical (HPA) axis dysfunction as opposed to playing an active role in the disease (28). Even if DHEAS does not play any direct role in the disease process itself, DHEAS administration may provide a means by which the deleterious effects of chronic glucocorticoid administration (29) could be minimised.

With regard to correlations between serum DHEAS concentrations and disease activity variables (ESR, CRP and tender joint count), we could only find a correlation between ESR at day 21 and DHEAS. This positive correlation is somewhat unexpected, as previous studies have found inverse correlations between serum DHEAS and IL-6 (2, 4) the latter of which is thought to determine ESR via fibrinogen production.

IL-6 levels, on the other hand, correlated with several disease activity variables which suggests that IL-6, but not DHEAS, is associated with disease activity. The finding that IL-6 correlated with cortisol at baseline and on day 21 of the fasting study may reflect the bi-directional association between IL-6 and the HPA axis (30). IL-6 can also act directly upon adrenal gland cells in concert with ACTH to stimulate the release of corticosterone (31). In accordance with this, we noted that one patient (female, 36 years, 1 year disease duration) in the ketogenic diet group had IL-6 levels above the assay limits at all time points (> 370 pg/ml) with correspondingly high cortisol levels (781, 1201 and 575 nmol/l at baseline, day 7 and day 21). Contrastingly, this patient, who had not been treated with glucocorticoids, had markedly lowered serum DHEAS levels (0.49, 0.88 and 0.47 mmol/l at corresponding time points), an observation which is in accordance with the concept of glucocorticoid/adrenal androgen dissociation in a subset of RA patients (1, 28).

Recently, Komaki *et al.* found decreases in the absolute numbers of lymphocytes and CD4+ cells in 10 patients with psychosomatic disorders after a 7 to 10 day total fast and suggested that these chan-

ges are at least partly due to fasting induced increases in DHEAS and cortisol (12). As we previously found marked decreases in total lymphocyte counts, CD4+ lymphocyte counts and mitogen stimulated CD4+ activation after fasting, but not in response to a ketogenic diet (11,14) our results here suggest that these changes are unlikely to be mediated by increases in serum DHEAS.

We must take into consideration that the fasting was carried out at a health farm whilst the ketogenic diet was carried out at home, which could place different physiological and psychological demands upon the patients. As stress may adversely affect RA (32), the effects obtained during fasting may have been associated with the stress-free environment associated with a health-farm stay. However, an earlier study carried out by Kjeldsen-Kragh *et al.*, does not support this hypothesis (10). In this study of the effects of fasting and diet upon RA, a control group of 26 patients were sent to a recreation home where they followed an omnivorous diet and received physiotherapy at a similar frequency as those patients who fasted at the health farm. In contrast to the fasting patients, no significant improvements in objective clinical and laboratory variables were found in the patients who attended the convalescent home, suggesting that fasting, rather than rest and relaxation, is responsible for the clinical improvements. This has also been shown by studies comparing the effects of RA patients either fasting or following a normal diet in hospitalised RA patients (7, 9).

It should also be mentioned that, although the data do not support the idea that acute increases in serum DHEAS of the magnitude which we observed have clinical significance, care should be taken when drawing negative conclusion when the number of patients is small. We must also take into account that we have 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, disease duration, baseline serum DHEAS or IL-6 concentrations,

the median baseline CRP and IL-6 values were lower in patients who followed the ketogenic diet.

DHEAS levels are influenced by a pre- or post-menopausal onset of RA. We therefore used a two-way analysis of variance model to assess whether this factor differentially affected the responses to either the ketogenic diet or fasting with regard to clinical variables, IL-6 and DHEAS. However, we did not find any statistically significant interactions which would suggest that pre- or post-menopausal onset of disease was a confounding factor in the results of our study.

Glucocorticoid therapy may also affect both IL-6 and DHEAS. To assess if prednisolone therapy might explain the differences between fasting and a ketogenic diet in terms of clinical responses and changes in IL-6, we removed the data for the 4 patients in the ketogenic diet study and the 1 patient in the fasting study who were taking prednisolone. Removal of these patients did not alter any of our findings in either the fasting or ketogenic diet groups.

In conclusion, we have shown that similar increases in serum DHEAS occur in response to both fasting and a ketogenic diet in RA patients, whilst only fasting decreases serum IL-6 levels. As DHEAS decreases the expression and activity of the IL-6 gene promoter (3), the increase we observed in serum DHEAS during fasting may have contributed to the fall in IL-6 and, in turn, the falls in CRP and ESR. However, as we observed no significant change in IL-6 in response to a ketogenic diet, despite a comparable increase in serum DHEAS, it would appear that other hormones and/or factors may have mediated the fasting-induced fall in serum IL-6.

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