Effect of Ramadan fasting in Saudi Arabia on serum bone profile and immunoglobulins

Suhard M. Bahijri, Ghada M. Ajabnoor, Anwar Borai, Jumana Y. Al-Aama and George P. Chrousos

Ther Adv Endocrinol Metab

2015, Vol. 6(5) 223–232

DOI: 10.1177/

2042018815594527

© The Author(s), 2015.

Reprints and permissions:

<http://www.sagepub.co.uk/journalsPermissions.nav>

Abstract

Background: Each year Muslims fast from dawn to sunset for 1 month (Ramadan). In Saudi Arabia, the sleep–wake cycle during Ramadan is severely disturbed and is associated with abolition of the circadian cortisol rhythm, exposing Saudis to continuously increased cortisol levels, which may influence the immune response. In addition to cortisol, sleep and fasting affect the secretion of parathyroid hormone (PTH) and hence bone metabolism.

Methods: Our objective was to investigate the effect of Ramadan type fasting on secretory patterns of PTH, markers of bone metabolism, and serum immunoglobulins. Blood samples from healthy young volunteers were collected at 9 a.m. and 9 p.m. (± 1 hour) before (Shaban) and 2 weeks into Ramadan. Calcium, phosphorus, magnesium, albumin, alkaline phosphatase, 25-OH vitamin D, intact PTH (iPTH), and immunoglobulin (Ig) A, M and G were measured.

Results: During Ramadan, evening-adjusted calcium was higher ($p = 0.036$) and phosphate lower ($p < 0.001$) than the corresponding morning value. Moreover, the Ramadan mean morning phosphate was higher and the evening level lower was than Shabaan values ($p = 0.010$ and $p < 0.001$, respectively), while mean iPTH level was decreased compared with the morning value ($p = 0.001$), and the evening mean during Shabaan ($p = 0.029$). Mean IgG concentration was significantly lower during Ramadan ($p = 0.003$ and $p = 0.021$ for morning and evening, respectively).

Conclusions: Changes in dietary practices during Ramadan modulated PTH secretion to a pattern which might be beneficial to bone health. Combined effects of fasting and disturbed sleep led to a noted decrease in IgG level. Therefore, a possible beneficial effect of fasting on bone turnover is combined with decreased immune response.

Keywords: bone metabolism, cortisol, immune response, immunoglobulins, PTH, Ramadan fasting, sleep–wake cycle

Introduction

Intermittent fasting has received much attention recently, with reports of beneficial effects on various organ systems coming from different research groups [Anson *et al.* 2003; Bales and Kraus, 2013; Goodrick *et al.* 1990; Heilbronn and Ravussin, 2003; Horne *et al.* 2008; Mattson *et al.* 2002; Mattson and Wan, 2005; Sohal and Weindruch, 1996; Varady and Hellerstein, 2007]. Islam is a religion practiced by about 2 billion individuals and requires its followers to fast from dawn to sunset for 1 month a year, a practice named Ramadan. Due to the widespread nature of Muslim countries, there are Muslims from many ethnicities, with Ramadan fasting subjected to cultural influences of the host country. The

Kingdom of Saudi Arabia is the center of the Islamic world. In recent decades, with increasing modernization, the sleep–wake cycle during Ramadan has been subjected to an almost complete nyctohemeral reversal compared with the rest of the year, with most residents staying up until dawn, sleeping a few hours (if any) before going to work, and sleeping again after work or when possible. This lifestyle during Ramadan is not seen in other countries and offers a unique opportunity to study the effects of circadianly disturbed sleeping/eating patterns on hormonal secretion and metabolism. We previously reported that this disturbance during Ramadan was associated with loss of the circadian rhythm and increased evening levels of cortisol, a hormone

Correspondence to:
Suhad M. Bahijri, PhD, FICN
Department of Clinical
Biochemistry, Faculty of
Medicine, Saudi Diabetes
Research Group, King
Fahd Medical Research
Center, King Abdulaziz
University, PO Box 4873,
Jeddah 21412, Saudi
Arabia
sbahijri@gmail.com

Ghada M. Ajabnoor, PhD
Department of Clinical
Biochemistry, Faculty of
Medicine, King Abdulaziz
University, Jeddah, Saudi
Arabia
Saudi Diabetes Research
Group, King Fahd Medical
Research Center, King
Abdulaziz University,
Jeddah, Saudi Arabia

Anwar Borai, PhD
Saudi Diabetes Research
Group, King Fahd Medical
Research Center, King
Abdulaziz University,
Jeddah, Saudi Arabia
King Abdullah
International Medical
Research Center, King
Saud bin Abdulaziz
University for Health
Sciences, King Abdulaziz
Medical City, Jeddah,
Saudi Arabia

Jumana Y. Al-Aama, MRCP, FCCMG
Saudi Diabetes Research
Group, King Fahd Medical
Research Center, King
Abdulaziz University,
Jeddah, Saudi Arabia
Department of Genetic
Medicine, Faculty of
Medicine, King Abdulaziz
University, Jeddah, Saudi
Arabia
Princess Al-Jawhara
Al Brahim Center of
Excellence in Research of
Hereditary Disorders

George P. Chrousos, MD, ScD

Saudi Diabetes Research Group, King Fahd Medical Research Center, King Abdulaziz University, Jeddah, Saudi Arabia
First Department of Pediatrics, University of Athens Medical School, 'Aghia Sophia' Children's Hospital, Athens, Greece

that controls the expression of many hormones and inflammatory mediators, which might have deleterious effects on metabolic homeostasis [Bahijri *et al.* 2013].

Subclinical hypercortisolism has been associated with signs of glucocorticoid-induced osteoporosis (GIOP) [Chiodini *et al.* 2002; Morelli *et al.* 2011; Tauchmanova *et al.* 2001] and increased frequency of subclinical hypercortisolism was reported among patients with established osteoporosis [Chiodini *et al.* 2007; Kann *et al.* 2001]. Hence, bone metabolism might be affected during Ramadan fasting. A hormone that is important for maintaining bone integrity is parathyroid hormone (PTH) [Kroll, 2000]. PTH exhibits circadian rhythmicity [Jubiz *et al.* 1972; Logue *et al.* 1989]. This physiological variation in the circulating levels of PTH has been linked to bone formation [Nielsen *et al.* 1991] and is affected by sleep [Kripke *et al.* 1978; Logue *et al.* 1992]. In addition, acute fasting was reported to result in a significant decrease in serum PTH, which was suggested to be secondary to increased bone resorption [Schlemmer and Hassager, 1999].

Another major function of cortisol is to modulate immune responses during stress [McEwen *et al.* 1997]. It suppresses the production and activity of many proinflammatory cytokines during stressful periods and helps to return the organism back to baseline (homeostasis) after cessation of the stressor [McEwen *et al.* 1997; McKay and Cidlowski, 1999; Ruzek *et al.* 1999]. Hence, effects on the immune system function are expected due to Ramadan fasting and disrupted pattern of cortisol secretion. Therefore, we aimed to investigate the effects of this type of religious fasting in Saudi Arabia and its associated disturbance in the sleep–wake cycle and feeding patterns on bone metabolism reflected on the concentrations of bone minerals (calcium, phosphorus and magnesium), alkaline phosphatase, vitamin D and PTH, as well as levels of immunoglobulin (Ig) A, M and G.

Subjects and methods

Study design

The study design, physiological measurements and anthropometry were described in detail in our earlier report [Bahijri *et al.* 2013] and are summarized below. Studying the same subjects at different times helped us to avoid group variability at the study baseline and in reducing the number of

subjects needed. Sample size was calculated to avoid type II statistical error [Eng, 2003] by employing reference ranges for intended measurements, as well as laboratory quality control data.

The study received approval from the Committee on the Ethics of Human Research at the Faculty of Medicine, King Abdulaziz University. Written informed consent was obtained in all cases. Of the recruited volunteer healthy subjects, 2 dropped out of the study, leaving 23 (18 males, 5 females), aged 18–42 years, to complete it. Volunteers were studied twice, during their regular life (Shaban) before, and again 10–15 days into the fasting period (Ramadan). All but one were <30 years of age, taking no medications or nutritional supplements; 3 subjects were obese [body mass index (BMI) ≥ 30 kg/m²], 4 were overweight (BMI 25 to <30) and the rest had a normal BMI (<25). One male subject of normal weight was found to have a slightly elevated blood pressure (154/94) before taking the second blood sample. To avoid effects of diet and sleeping patterns on measured parameters, subjects were instructed to have meals as usual on the day of testing and to record their usual sleeping and waking times for the previous 3 days.

Blood samples were drawn twice daily at 9 a.m. $\pm$ 1 hour and again 12 hours later. Thus, the first and third samples were obtained while fasting (at least 10 hours for the first sample and 6–7 hours for the third sample), while the second and fourth samples were obtained 2–5 hours after meals. Separated serum samples were stored at -80°C until measurements were performed.

Biochemical and endocrine assays

All serum biochemical and endocrine parameters were assayed in the accredited clinical chemistry laboratory at the National Guard Hospital, King Abdulaziz Medical City-Jeddah, Kingdom of Saudi Arabia. Calcium, albumin, magnesium, inorganic phosphorous and total alkaline phosphatase were assayed by a spectrophotometric method, while IgG, IgM and IgA were measured using an immunoturbidimetric method. An Abbott Architect c8000 auto-analyzer was used. An Abbott Architect i2000 auto-analyzer was used to measure intact PTH (iPTH) and total 25-OH vitamin D using a two-step sandwich chemiluminescent microparticle immunoassay (CMIA) technique.

Statistical analyses. Analyses were performed using SPSS statistical package version 19.

Descriptive statistics, such as mean $\pm$ standard error of the mean (SEM), were calculated for all estimated parameters. The paired Student's *t*-test was employed for comparison of means between days and nights. Significance was assigned at $p < 0.05$.

Results

The results of estimated biochemical, endocrine and immunological parameters are presented in Table 1, Figure 1 and Figure 2. No significant differences between morning and evening albumin concentration were noted during Shaban or Ramadan. However, both morning and evening Ramadan levels were significantly decreased compared with pre fast values ($p = 0.001$ in both cases) (Table 1).

After correction for albumin concentration, evening-adjusted calcium was higher only during Ramadan ($p = 0.036$), with no significant differences between Shaban and Ramadan values in the morning or in the evening (Figure 1). In contrast, evening phosphate level was significantly lower than the morning value during Ramadan only ($p < 0.001$). In addition, during Ramadan, the morning level was higher and the evening level was lower than the Shaban corresponding

values ($p = 0.010$ and $p < 0.001$, respectively) (Figure 1). Associated with these changes, there was a significant decrease in the evening mean level of circulating iPTH during Ramadan compared with the morning mean level ($p = 0.001$) and compared with the evening mean values during Shaban ($p = 0.029$) (Figure 2). In addition, during Shaban three subjects had slightly elevated concentrations of the hormone either in the morning or the evening sample, and two had more than double the top normal range in the morning and evening. During Ramadan, a slight elevation was noted for four subjects in the morning samples and for one subject in the evening sample. Only one subject with more than double the top normal range values during Shaban continued to have an elevated level during Ramadan.

According to the latest suggested classification of vitamin D status [Thacher and Clarke, 2011], 4 subjects had 25-OH vitamin D levels below the recognized severe deficiency cutoff level of ≤ 10 ng/ml or ≤ 25 nmol/l during Shaban, with 2 more falling into this category during Ramadan. However, during Shaban and Ramadan, the remaining subjects were in the vitamin D insufficiency range (> 10 – 20 ng or > 25 to < 50 nmol/l) and none had serum concentrations ≥ 30 ng/ml or 75 nmol/l, suggested as the cutoff value for

Table 1. Serum biochemical, endocrine and immunological parameters during Shaban and Ramadan.

		Shaban		Ramadan		Shaban versus Ramadan
		Mean $\pm$ SEM	<i>p</i> value [AM–PM]	Mean $\pm$ SEM	<i>p</i> value [AM–PM]	<i>p</i> value
Magnesium (mmol/l)	AM	0.80 $\pm$ 0.01	0.626	0.77 $\pm$ 0.02	0.078	0.081
	PM	0.81 $\pm$ 0.01		0.85 $\pm$ 0.04		0.179
Calcium (mmol/l)	AM	2.41 $\pm$ 0.02	<0.001	2.34 $\pm$ 0.03	<0.001	0.025
	PM	2.50 $\pm$ 0.02		2.51 $\pm$ 0.02		0.467
Albumin (g/l)	AM	42.0 $\pm$ 0.5	0.713	40.1 $\pm$ 0.4	0.697	0.001*
	PM	41.9 $\pm$ 0.6		40.0 $\pm$ 0.4		0.001
ALP (U/l)	AM	79.8 $\pm$ 7.0		75.5 $\pm$ 6.9		0.199*
25-OH vitamin D (nmol/l)	AM	33.96 $\pm$ 2.92		32.11 $\pm$ 2.84		0.111
IgM (g/l)	AM	0.873 $\pm$ 0.099	0.121	0.873 $\pm$ 0.124	0.926	0.212
	PM	0.893 $\pm$ 0.097		0.945 $\pm$ 0.122		0.839
IgA (g/l)	AM	1.86 $\pm$ 0.14	0.025	1.85 $\pm$ 0.16	<0.001	0.607
	PM	1.91 $\pm$ 0.14		2.10 $\pm$ 0.15		0.660
IgG (g/l)	AM	12.37 $\pm$ 0.44	0.040	11.33 $\pm$ 0.34	<0.001	0.003
	PM	12.67 $\pm$ 0.41		12.56 $\pm$ 0.42		0.021

*Reported previously [Ajabnoor *et al.* 2014].
ALP, alkaline phosphatase; Ig, immunoglobulin; SEM, standard error of the mean.
Note: significant differences are indicated in bold

Figure 1. Serum morning and evening phosphate and adjusted calcium concentrations during Shaban and Ramadan.

Figure 2. Serum morning and evening intact parathyroid hormone (iPTH) concentration during Shaban and Ramadan.

defining optimal vitamin D status [Kennel *et al.* 2010; Prentice *et al.* 2008]. No significant difference was found in mean concentration of 25-OH vitamin D between Shaban and Ramadan values.

IgM mean concentrations did not show diurnal variation, neither was there a difference between means in Shaban and corresponding Ramadan

means. However, means of IgA and IgG showed a significant increase in the evening during Shaban and Ramadan, with those of IgG being significantly lower during Ramadan than their corresponding Shaban means (Table 1).

No significant changes were noted in the means of other estimated parameters, and all values were within normal reference ranges.

Discussion

In normal healthy subjects, adequate sleep quality and duration associated with intermittent fasting, even without or little overall decrease in calorie intake, has been reported to increase lifespan [Anson *et al.* 2003; Goodrick *et al.* 1990] and improve health [Heilbronn and Ravussin, 2003; Mattson *et al.* 2002; Sohal and Weindruch, 1996]. However, studies on Muslims practicing Ramadan fasting gave conflicting results, with some reporting beneficial effects on metabolism [Adlouni *et al.* 1997, 1998; Maislos *et al.* 1993], and others reporting increased metabolic and endocrine dysregulation [Chennaoui *et al.* 2009; Fedail *et al.* 1982; Gumaa *et al.* 1978]. The Islamic world extends over a wide range of geographic locations, with different dietary habits and social practices. Therefore, studies carried out in different locations on Ramadan practitioners are likely to produce different results [Chandalia *et al.* 1987; El Ati *et al.* 1995].

Short-term fasting was reported to cause a significant decline in bone formation, independently of changes in acid–base status [Grinspoon *et al.* 1995]. Furthermore, changing dietary patterns were reported to have an effect on bone turnover in the rat, with division of intake into portions leading to decreased resorption [Muhlbauer and Fleisch, 1995]. In addition, there is circadian variation of biochemical markers of bone formation [Eastell *et al.* 1992b; Hassager *et al.* 1992; Nielsen *et al.* 1990; Pedersen *et al.* 1995] and is thought to be dependent on the circadian fluctuation in serum cortisol [Nielsen *et al.* 1988, 1992; Schlemmer *et al.* 1997]. Furthermore, biochemical parameters of bone resorption also show circadian variation, with the peak during the night/early morning and the nadir late in the afternoon [Schlemmer *et al.* 1994]. Therefore, in view of the high prevalence of osteoporosis in Saudi Arabia [El-Desouki, 2003; El-Desouki and Sulimani, 2007; Sadat-Ali *et al.* 2012], we considered it is important to study the effects of Ramadan type fasting, with its associated disruption of sleep/wake cycle and feeding pattern, in the Kingdom. We evaluated biochemical markers commonly used to study bone metabolism, namely 25-OHvitamin D, PTH, alkaline phosphatase and the bone minerals calcium, phosphorus and magnesium.

Total vitamin D levels were not affected by fasting and disturbed sleep pattern, as the mean 25-OH vitamin D level did not change significantly.

Hence, noted changes in calcium and phosphate concentration are likely to be due to other factors such as changes in PTH concentration and feeding patterns.

PTH plays an important role in bone turnover, with studies indicating that continuous hypersecretion of PTH is associated with bone resorption, while intermittent administration of the hormone at a low dose has a positive effect on bone volume and micro-architecture [Kroll, 2000]. Moreover, changes in the circadian rhythms of PTH were reported in patients with postmenopausal osteoporosis [Fraser *et al.* 1998]. Thus, manipulation of PTH secretion has been suggested as a way of increasing bone strength and treating osteoporosis [Fraser *et al.* 2004; Silver and Bushinsky, 2004]. The impact of sleep on the circadian pattern of PTH is controversial [Kripke *et al.* 1978; Logue *et al.* 1992], with one study reporting that a large component of its diurnal rhythm is not effected by sleep–wake cycles [El-Hajj Fuleihan *et al.* 1997]. Normally, PTH peaks in the early morning hours, with a nadir in the late morning before noon [Kitamura *et al.* 1990], a secondary peak in the late afternoon, and a secondary nadir in the late evening before midnight. In our study, sampling was performed at 9 a.m. $\pm$ 1 hour, and again 12 hours later; hence higher levels were expected in the morning samples. Indeed, this was found both during Shaban (pre-fasting) and Ramadan, with the latter reaching statistical significance ($p = 0.001$). Thus, sleep disturbance did not seem to have a marked effect on the circadian rhythm of the hormone, most likely due to the reported sleep-independent endogenous nature of the rhythm [El-Hajj Fuleihan *et al.* 1997]. However, the noted increased difference between morning and evening concentration during Ramadan could be beneficial to bone health, since the amplitude in the diurnal rhythm of intact PTH levels was reported to be greater in normal subjects than in osteoporotic subjects [Eastell *et al.* 1992a].

Fasting is reported to decrease the circulating PTH concentration [Schlemmer and Hassager, 1999]. Ramadan iPTH values were lower than corresponding Shabaan values, with evening mean concentration being significantly lower ($p = 0.029$) and fewer subjects having higher than normal levels. This could be attributed to fasting and the altered feeding pattern. During Ramadan, food (including dairy products) was ingested 2–3

hours before taking the evening sample; hence, the lower iPTH mean concentration corresponded with an increased post absorptive calcium concentration and a decreased phosphate concentration with increased carbohydrate metabolism (Figures 1 and 2), both acting to repress PTH secretion. Thus, it seems that the change in dietary patterns during Ramadan helps modulate PTH secretion by increasing evening calcium concentration, hence decreasing bone resorption during the night. Furthermore, changes in measured parameters are not due to fasting leading to hemoconcentration (dehydration) or acidosis, granted that electrolyte concentrations in the morning and evening samples did not change significantly during Shaban and Ramadan, as reported earlier [Bahijri *et al.* 2013].

Both factors of fasting and sleep deprivation have an effect on immune system function and responses. Debate is still ongoing regarding the benefits of fasting in relation to health and immunity [Varady and Hellerstein, 2007]. Both the specific and non-specific immune responses are affected by fasting and the effect is dependent on age [Walrand *et al.* 2001] and nutritional status [McMurray *et al.* 1990; Wing *et al.* 1983]. During fasting, the number of B lymphocytes was significantly decreased, but an increase was described after the refeeding period, which was suggested to promote Ig production and to contribute to the induction of immune function after meal absorption [Walrand *et al.* 2001]. The effects on immune variables were attributed at least in part to changes in adrenal gland-related hormones [Komaki *et al.* 1997].

Ramadan type fasting modulates the levels of some cytokines involved in the regulation of the immune response [Mohammed and Mahmood, 2010]. Serum IgA, IgG and IgM levels in healthy Iraqi subjects and in patients with type 2 diabetes mellitus were reported to be slightly decreased towards the end of Ramadan [Al-Jewari *et al.* 2007]. Another study on healthy adult Turkish males found decreased concentrations of serum IgG and salivary IgA during Ramadan compared with pre-fast levels, but the IgG concentration remained within the normal range [Develioglu *et al.* 2013]. However, a study on Tunisian judo athletes maintaining training during Ramadan found increased IgA, which remained high 3 weeks later, with no change in leukocyte count [Chaouachi *et al.* 2009]. Those studies did not include the effects of sleep disturbance and depri-

vation factors which are common in Saudi population during Ramadan.

Studies indicate that sleep deprivation has deleterious effects on regulation of cytokine production and immune cell function [Bryant *et al.* 2004]. Sleep deprivation in rats increased the production of IgM [Everson, 2005; Zager *et al.* 2007] and IgG [Everson, 2005; Gumustekin *et al.* 2004] and IgA [Everson, 2005]. In humans, 24 hours of sleep deprivation increased the level of serum IgG and IgA [Hui *et al.* 2007]. Similarly, in another study on healthy young males, sleep deprivation due to land travel increased the concentration of serum IgG and IgA, but had no significant effect on IgM concentration [Abolhasan *et al.* 2012]. This was associated with a decrease in serum cortisol. In contrast to these reports, decreased sleep duration lowered the antibody response to various vaccines [Benedict *et al.* 2012; Lange *et al.* 2011; Prather *et al.* 2012; Spiegel *et al.* 2002] and to increase susceptibility to infection [Cohen *et al.* 2009; Mohren *et al.* 2002].

A close link between sleep deprivation and activity of the hypothalamic–pituitary–adrenal (HPA) axis and the circulating concentrations of cortisol has been reported [Wu, 2008]. In our study, the effects of disturbed sleep–wake cycle and sleep deprivation was combined with the effects of fasting, resulting in an altered cortisol secretion pattern as reported earlier [Bahijri *et al.* 2013] and leading to the noted significant decrease in IgG level during Ramadan (Table 1) ($p = 0.003$, and 0.021 for morning and evening values, respectively). Thus, subjects practicing Ramadan fasting in Saudi Arabia might have an increased risk of infection and a lower response to vaccines. Therefore, more studies are required to investigate the immune system status during Ramadan.

In conclusion, Ramadan fasting in Saudi Arabia might have some beneficial effects on bone turnover; however, it is associated with a decrease in the immune response. This should be considered when planning activities during this month, especially in the elderly, and chronically diseased or malnourished individuals.

Acknowledgements

The authors acknowledge with thanks DSR technical and financial support. The authors would also like to thank the Abuzinadah Hospital in Jeddah for help with sample collection.

Funding

The author(s) disclosed receipt of the following financial support for the research, authorship, and/or publication of this article: This project was funded by the Deanship of Scientific Research (DSR) at King Abdulaziz University, Jeddah, Kingdom of Saudi Arabia under grant number (HiCi/1432- 6-2) as part of research activities conducted by the Saudi Diabetes Study Research Group.

Declaration of Conflicting Interests

The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

References

- Abolhasan, H., Peeri, M., Azarbayjani, M. and Mazidi, A. (2012) The effect of sleep deprivation due to land travel on the concentration of immunoglobulins A, G, and M and serum cortisol in young soccer players. *Ann Biol Res* 3: 1255–1263.
- Adlouni, A., Ghalim, N., Benslimane, A., Lecerf, J. and Saile, R. (1997) Fasting during Ramadan induces a marked increase in high-density lipoprotein cholesterol and decrease in low-density lipoprotein cholesterol. *Ann Nutr Metab* 41: 242–249.
- Adlouni, A., Ghalim, N., Saile, R., Hda, N., Parra, H. and Benslimane, A. (1998) Beneficial effect on serum apo AI, apo B and Lp AI levels of Ramadan fasting. *Clin Chim Acta* 271: 179–189.
- Ajabnoor, G., Bahijri, S., Borai, A., Abdulkhalik, A., Al-Aama, J. and Chrousos, G. P. (2014) Health impact of fasting in Saudi Arabia during Ramadan: association with disturbed circadian rhythm and metabolic and sleeping patterns. *PLoS One* 9: e96500.
- Al-Jewari, M., Mohammed, K. and Al-Hakim, S. (2007) Effect of Ramadan fasting on clinical biochemical and immunological parameters in healthy fasting and type 2 diabetes mellitus patients. *Iraqi Postgrad Med J* 6: 272–275.
- Anson, R., Guo, Z., de Cabo, R., Iyun, T., Rios, M., Hagepanos, A. *et al.* (2003) Intermittent fasting dissociates beneficial effects of dietary restriction on glucose metabolism and neuronal resistance to injury from calorie intake. *Proc Natl Acad Sci U S A* 100: 6216–6220.
- Bahijri, S., Borai, A., Ajabnoor, G., Abdul Khaliq, A., Alqassas, I. Al-Shehri, D. *et al.* (2013) Correction: Relative metabolic stability, but disrupted circadian cortisol secretion during the fasting month of Ramadan. *PLoS One*. DOI: 10.1371/annotation/8d92315c-9944-4470-9bbb-806a26b0809b.
- Bales, C. and Kraus, W. (2013) Caloric restriction: implications for human cardiometabolic health. *J Cardiopulm Rehabil Prev* 33: 201–208.
- Benedict, C., Brytting, M., Markstrom, A., Broman, J. and Schioth, H. (2012) Acute sleep deprivation has no lasting effects on the human antibody titer response following a novel influenza A H1N1 virus vaccination. *BMC Immunol* 13: 1.
- Bryant, P., Trinder, J. and Curtis, N. (2004) Sick and tired: does sleep have a vital role in the immune system? *Nat Rev Immunol* 4: 457–467.
- Chandalia, H., Bhargava, A. and Kataria, V. (1987) Dietary pattern during Ramadan fasting and its effect on the metabolic control of diabetes. *Pract Diabetes Int* 4: 287–290.
- Chaouachi, A., Coutts, A., del Wong, P., Roky, R., Mbazaa, A., Amri, M. *et al.* (2009) Haematological, inflammatory, and immunological responses in elite judo athletes maintaining high training loads during Ramadan. *Appl Physiol Nutr Metab* 34: 907–915.
- Chennaoui, M., Desgorges, F., Drogou, C., Boudjemaa, B., Tomaszewski, A., Depiesse, F. *et al.* (2009) Effects of Ramadan fasting on physical performance and metabolic, hormonal, and inflammatory parameters in middle-distance runners. *Appl Physiol Nutr Metab* 34: 587–594.
- Chiodini, I., Mascia, M., Muscarella, S., Battista, C., Minisola, S., Arosio, M. *et al.* (2007) Subclinical hypercortisolism among outpatients referred for osteoporosis. *Ann Intern Med* 147: 541–548.
- Chiodini, I., Tauchmanova, L., Torlontano, M., Battista, C., Guglielmi, G., Cammisa, M. *et al.* (2002) Bone involvement in eugonadal male patients with adrenal incidentaloma and subclinical hypercortisolism. *J Clin Endocrinol Metab* 87: 5491–5494.
- Cohen, S., Doyle, W., Alper, C., Janicki-Deverts, D. and Turner, R. (2009) Sleep habits and susceptibility to the common cold. *Arch Intern Med* 169: 62–67.
- Develioglu, O., Kucur, M., Ipek, H., Celebi, S., Can, G. and Kulekci, M. (2013) Effects of Ramadan fasting on serum immunoglobulin G and M, and salivary immunoglobulin A concentrations. *J Int Med Res* 41: 463–472.
- Eastell, R., Calvo, M., Burritt, M., Offord, K., Russell, R. and Riggs, B.L. (1992a) Abnormalities in circadian patterns of bone resorption and renal calcium conservation in type I osteoporosis. *J Clin Endocrinol Metab* 74: 487–494.
- Eastell, R., Simmons, P., Colwell, A., Assiri, A., Burritt, M., Russell, R. G. G. *et al.* (1992b) Nyctohemeral changes in bone turnover assessed by serum bone Gla-protein concentration and urinary

- deoxyypyridinoline excretion: effects of growth and ageing. *Clin Sci* 83: 375–382.
- El-Desouki, M. (2003) Osteoporosis in postmenopausal Saudi women using dual X-ray bone densitometry. *Saudi Med J* 24: 953–956.
- El-Desouki, M. and Sulimani, R. (2007) High prevalence of osteoporosis in Saudi men. *Saudi Med J* 28: 774–777.
- El-Hajj Fuleihan, G., Klerman, E., Brown, E., Choe, Y., Brown, E. and Czeisler, C. A. (1997) The parathyroid hormone circadian rhythm is truly endogenous—a general clinical research center study. *J Clin Endocrinol Metab* 82: 281–286.
- El Ati, J., Beji, C. and Danguir, J. (1995) Increased fat oxidation during Ramadan fasting in healthy women: an adaptative mechanism for body-weight maintenance. *Am J Clin Nutr* 62: 302–307.
- Eng, J. (2003) Sample size estimation: how many individuals should be studied? *Radiology* 227: 309–313.
- Everson, C. (2005) Clinical assessment of blood leukocytes, serum cytokines, and serum immunoglobulins as responses to sleep deprivation in laboratory rats. *Am J Physiol Regul Integr Comp Physiol* 289: R1054–R1063.
- Fedail, S., Murphy, D., Salih, S., Bolton, C. and Harvey, R. (1982) Changes in certain blood constituents during Ramadan. *Am J Clin Nutr* 36: 350–353.
- Fraser, W., Ahmad, A. and Vora, J. (2004) The physiology of the circadian rhythm of parathyroid hormone and its potential as a treatment for osteoporosis. *Curr Opin Nephrol Hypertens* 13: 437–444.
- Fraser, W., Logue, F., Christie, J., Gallacher, S., Cameron, D., O'Reilly, D. *et al.* (1998) Alteration of the circadian rhythm of intact parathyroid hormone and serum phosphate in women with established postmenopausal osteoporosis. *Osteoporos Int* 8: 121–126.
- Goodrick, C., Ingram, D., Reynolds, M., Freeman, J. and Cider, N. (1990) Effects of intermittent feeding upon body weight and lifespan in inbred mice: interaction of genotype and age. *Mech Ageing Dev* 55: 69–87.
- Grinspoon, S., Baum, H., Kim, V., Coggins, C. and Klibanski, A. (1995) Decreased bone formation and increased mineral dissolution during acute fasting in young women. *J Clin Endocrinol Metab* 80: 3628–3633.
- Gumaa, K., Mustafa, K., Mahmoud, N. and Gader, A. (1978) The effects of fasting in Ramadan. 1. Serum uric acid and lipid concentrations. *Br J Nutr* 40: 573–581.
- Gumustekin, K., Seven, B., Karabulut, N., Aktas, O., Gursan, N., Aslan, S. *et al.* (2004) Effects of sleep deprivation, nicotine, and selenium on wound healing in rats. *Int J Neurosci* 114: 1433–1442.
- Hassager, C., Risteli, J., Risteli, L., Jensen, S. and Christiansen, C. (1992) Diurnal variation in serum markers of type I collagen synthesis and degradation in healthy premenopausal women. *J Bone Miner Res* 7: 1307–1311.
- Heilbronn, L. and Ravussin, E. (2003) Calorie restriction and aging: review of the literature and implications for studies in humans. *Am J Clin Nutr* 78: 361–369.
- Horne, B., May, H., Anderson, J., Kfoury, A., Bailey, B., McClure, B. S. *et al.* (2008) Usefulness of routine periodic fasting to lower risk of coronary artery disease in patients undergoing coronary angiography. *Am J Cardiol* 102: 814–819.
- Hui, L., Hua, F., Diandong, H. and Hong, Y. (2007) Effects of sleep and sleep deprivation on immunoglobulins and complement in humans. *Brain Behav Immun* 21: 308–310.
- Jubiz, W., Canterbury, J., Reiss, E. and Tyler, F. (1972) Circadian rhythm in serum parathyroid hormone concentration in human subjects: correlation with serum calcium, phosphate, albumin, and growth hormone levels. *J Clin Invest* 51: 2040–2046.
- Kann, P., Laudes, M., Piepkorn, B., Heintz, A. and Beyer, J. (2001) Suppressed levels of serum cortisol following high-dose oral dexamethasone administration differ between healthy postmenopausal females and patients with established primary vertebral osteoporosis. *Clin Rheumatol* 20: 25–29.
- Kennel, K., Drake, M. and Hurley, D. (2010) Vitamin D deficiency in adults: when to test and how to treat. *Mayo Clin Proc* 85: 752–758.
- Kitamura, N., Shigeno, C., Shiomi, K., Lee, K., Ohta, S., Sone, T. *et al.* (1990) Episodic fluctuation in serum intact parathyroid hormone concentration in men. *J Clin Endocrinol Metab* 70: 252–263.
- Komaki, G., Kanazawa, F., Sogawa, H., Mine, K., Tamai, H., Okamura, S. *et al.* 1997. Alterations in lymphocyte subsets and pituitary-adrenal gland-related hormones during fasting. *Am J Clin Nutr* 66: 147–152.
- Kripke, D., Lavie, P., Parker, D., Huey, L. and Deftos, L. (1978) Plasma parathyroid hormone and calcium are related to sleep stage cycles. *J Clin Endocrinol Metab* 47: 1021–1027.
- Kroll, M. (2000) Parathyroid hormone temporal effects on bone formation and resorption. *Bull Math Biol* 62: 163–188.

- Lange, T., Dimitrov, S., Bollinger, T., Dieckmann, S. and Born, J. (2011) Sleep after vaccination boosts immunological memory. *J Immunol* 187: 283–290.
- Logue, F., Fraser, W., O'Reilly, D. and Beastall, G. (1989) The circadian rhythm of intact parathyroid hormone (1–84) and nephrogenous cyclic adenosine monophosphate in normal men. *J Endocrinol* 121: R1–R3.
- Logue, F., Fraser, W., O'Reilly, D., Christie, J., Cameron, D. and Beastall, G. H. (1992) Sleep shift dissociates the nocturnal peaks of parathyroid hormone (1–84), nephrogenous cyclic adenosine monophosphate, and prolactin in normal men. *J Clin Endocrinol Metab* 75: 25–29.
- Maislos, M., Khamaysi, N., Assali, A., Abou-Rabiah, Y., Zvili, I. and Shany, S. (1993) Marked increase in plasma high-density-lipoprotein cholesterol after prolonged fasting during Ramadan. *Am J Clin Nutr* 57: 640–642.
- Mattson, M., Chan, S. and Duan, W. (2002) Modification of brain aging and neurodegenerative disorders by genes, diet, and behavior. *Physiol Rev* 82: 637–672.
- Mattson, M. and Wan, R. (2005) Beneficial effects of intermittent fasting and caloric restriction on the cardiovascular and cerebrovascular systems. *J Nutr Biochem* 16: 129–137.
- McEwen, B., Biron, C., Brunson, K., Bulloch, K., Chambers, W., Dhabhar, F.S. (1997) The role of adrenocorticoids as modulators of immune function in health and disease: neural, endocrine and immune interactions. *Brain Res Brain Res Rev* 23: 79–133.
- McKay, L. and Cidlowski, J. (1999) Molecular control of immune/inflammatory responses: interactions between nuclear factor-kappa B and steroid receptor-signaling pathways. *Endocr Rev* 20: 435–459.
- McMurray, R., Bradsher, R., Steele, R. and Pilkington, N. (1990) Effect of prolonged modified fasting in obese persons on in vitro markers of immunity: lymphocyte function and serum effects on normal neutrophils. *Am J Med Sci* 299: 379–385.
- Mohammed, K. and Mahmood, M. (2010) Effect of Ramadan fasting on the levels of IL-1a, IL-2, IL-6 and IL-8 cytokines. *Diyala J Pure Sci* 6: 308–313.
- Mohren, D., Jansen, N., Kant, I., Galama, J., van den Brandt, P. and Swaen, G.M. (2002) Prevalence of common infections among employees in different work schedules. *J Occup Environ Med* 44: 1003–1011.
- Morelli, V., Eller-Vainicher, C., Salcuni, A., Coletti, F., Iorio, L., Muscogiuri, G. *et al.* (2011) Risk of new vertebral fractures in patients with adrenal incidentaloma with and without subclinical hypercortisolism: a multicenter longitudinal study. *J Bone Miner Res* 26: 1816–1821.
- Muhlbauer, R. and Fleisch, H. (1995) The diurnal rhythm of bone resorption in the rat. Effect of feeding habits and pharmacological inhibitors. *J Clin Invest* 95: 1933–1940.
- Nielsen, H., Brixen, K. and Mosekilde, L. (1990) Diurnal rhythm and 24-hour integrated concentrations of serum osteocalcin in normals: influence of age, sex, season, and smoking habits. *Calcif Tissue Int* 47: 284–290.
- Nielsen, H., Brixen, K., Kassem, M., Charles, P. and Mosekilde, L. (1992) Inhibition of the morning cortisol peak abolishes the expected morning decrease in serum osteocalcin in normal males: evidence of a controlling effect of serum cortisol on the circadian rhythm in serum osteocalcin. *J Clin Endocrinol Metab* 74: 1410–1414.
- Nielsen, H., Brixen, K., Kassem, M., Christensen, S. and Mosekilde, L. (1991) Diurnal rhythm in serum osteocalcin: relation with sleep, growth hormone, and PTH(1–84). *Calcif Tissue Int* 49: 373–377.
- Nielsen, H., Charles, P. and Mosekilde, L. (1988) The effect of single oral doses of prednisone on the circadian rhythm of serum osteocalcin in normal subjects. *J Clin Endocrinol Metab* 67: 1025–1030.
- Pedersen, B., Schlemmer, A., Rosenquist, C., Hassager, C. and Christiansen, C. (1995) Circadian rhythm in type I collagen formation in postmenopausal women with and without osteopenia. *Osteoporos Int* 5: 472–477.
- Prather, A., Hall, M., Fury, J., Ross, D., Muldoon, M., Cohen, S. *et al.* (2012) Sleep and antibody response to hepatitis B vaccination. *Sleep* 35: 1063–1069.
- Prentice, A., Goldberg, G. and Schoenmakers, I. (2008) Vitamin D across the lifecycle: physiology and biomarkers. *Am J Clin Nutr* 88: 500S–506S.
- Ruzek, M., Pearce, B., Miller, A. and Biron, C. (1999) Endogenous glucocorticoids protect against cytokine-mediated lethality during viral infection. *J Immunol* 162: 3527–3533.
- Sadat-Ali, M., Al-Habdan, I., Al-Turki, H. and Azam, M. (2012) An epidemiological analysis of the incidence of osteoporosis and osteoporosis-related fractures among the Saudi Arabian population. *Ann Saudi Med* 32: 637–641.
- Schlemmer, A. and Hassager, C. (1999) Acute fasting diminishes the circadian rhythm of biochemical markers of bone resorption. *Eur J Endocrinol* 140: 332–337.
- Schlemmer, A., Hassager, C., Alexandersen, P., Fledelius, C., Pedersen, B., Kristensen, J. *et al.* (1997)

- Circadian variation in bone resorption is not related to serum cortisol. *Bone* 21: 83–88.
- Schlemmer, A., Hassager, C., Pedersen, B. and Christiansen, C. (1994) Posture, age, menopause, and osteopenia do not influence the circadian variation in the urinary excretion of pyridinium crosslinks. *J Bone Miner Res* 9: 1883–1888.
- Silver, J. and Bushinsky, D. (2004) Harnessing the parathyroids to create stronger bones. *Curr Opin Nephrol Hypertens* 13: 471–476.
- Sohal, R. and Weindruch, R. (1996) Oxidative stress, caloric restriction, and aging. *Science* 273: 59–63.
- Spiegel, K., Sheridan, J. and Van Cauter, E. (2002) Effect of sleep deprivation on response to immunization. *JAMA* 288: 1471–1472.
- Tauchmanova, L., Rossi, R., Nuzzo, V., del Puente, A., Esposito-del Puente, A., Pizzi, C. *et al.* (2001) Bone loss determined by quantitative ultrasonometry correlates inversely with disease activity in patients with endogenous glucocorticoid excess due to adrenal mass. *Eur J Endocrinol* 145: 241–247.
- Thacher, T. and Clarke, B. (2011) Vitamin D insufficiency. *Mayo Clin Proc* 86: 50–60.
- Varady, K. and Hellerstein, M. (2007) Alternate-day fasting and chronic disease prevention: a review of human and animal trials. *Am J Clin Nutr* 86: 7–13.
- Walrand, S., Moreau, K., Caldefie, F., Tridon, A., Chassagne, J., Portefaix, G. *et al.* (2001) Specific and nonspecific immune responses to fasting and refeeding differ in healthy young adult and elderly persons. *Am J Clin Nutr* 74: 670–678.
- Wing, E., Stanko, R., Winkelstein, A. and Adibi, S. (1983) Fasting-enhanced immune effector mechanisms in obese subjects. *Am J Med* 75: 91–96.
- Wu, H., Zhao, Z., Stone, W., Huang, L., Zhuang, J., He, B., Zhang, P. *et al.* (2008) Effects of sleep restriction periods on serum cortisol levels in healthy men. *Brain Res Bull* 77: 241–245.
- Zager, A., Andersen, M., Ruiz, F., Antunes, I. and Tufik, S. (2007) Effects of acute and chronic sleep loss on immune modulation of rats. *Am J Physiol Regul Integr Comp Physiol* 293: R504–R509.