British Journal of Nutrition (2006), **96**, 1087–1094
DOI: 10.1017/BJN20061942
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https://doi.org/10.1017/BJN20061942 Published online by Cambridge University Press

Influence of Ramadan-type fasting on enzymes of carbohydrate metabolism and brush border membrane in small intestine and liver of rat used as a model

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(Received 6 February 2006 – Revised 3 July 2006 – Accepted 17 July 2006)

During Ramadan, Muslims the world over abstain from food and water from dawn to sunset for a month. We hypothesised that this unique model of prolonged intermittent fasting would result in specific intestinal and liver metabolic adaptations and hence alter metabolic activities. The effect of Ramadan-type fasting was studied on enzymes of carbohydrate metabolism and the brush border membrane of intestine and liver from rat used as a model. Rats were fasted (12 h) and then refed (12 h) daily for 30 d, as practised by Muslims during Ramadan. Ramadan-type fasting caused a significant decline in serum glucose, cholesterol and lactate dehydrogenase activity, whereas inorganic phosphate increased but blood urea N was not changed. Fasting resulted in increased activities of intestinal lactate (+34 %), isocitrate (+63 %), succinate (+83 %) and malate (+106 %) dehydrogenases, fructose 1,6-bisphosphatase (+17 %) and glucose-6-phosphatase (+22 %). Liver lactate dehydrogenase, malate dehydrogenase, glucose-6-phosphatase and fructose 1,6-bisphosphatase activities were also enhanced. However, the activities of glucose-6-phosphate dehydrogenase and malic enzyme fell significantly in the intestine but increased in liver. Although the activities of alkaline phosphatase, γ-glutamyl transpeptidase and sucrase decreased in mucosal homogenates and brush border membrane, those of liver alkaline phosphatase, γ-glutamyl transpeptidase and leucine aminopeptidase significantly increased. These changes were due to a respective decrease and increase of the maximal velocities of the enzyme reactions. Ramadan-type fasting caused similar effects whether the rats fasted with a daytime or night-time feeding schedule. The present results show a tremendous adaptation capacity of both liver and intestinal metabolic activities with Ramadan-type fasting in rats used as a model for Ramadan fasting in people.

Ramadan fasting: Carbohydrate metabolism: Intestine: Liver: Brush border membrane enzymes

The digestion and absorption of food components are major functions of the intestinal mucosa. These functions are dramatically altered by dietary status, including fasting, restricted energy intake and other dietary manipulations (Budhoski *et al*. 1982; Mayhew, 1987; Dou *et al*. 2001; Martins *et al*. 2001). Short-term fasting for a few days causes a significant decrease in glucose degradation with a concomitant increase in its production in the intestine and other tissues (Shen & Mistry, 1979; Farooq *et al*. 2004), whereas refeeding fasted rats resulted in a reversal of these effects (Buts *et al*. 1990).

Ramadan fasting is a unique model of fasting that is quite different from widely studied total fasting or starvation (Malhotra *et al*. 1989; Nomani *et al*. 1989; Cheah *et al.* 1990). During the Islamic month of Ramadan, which lasts for 29 or 30 d each year, millions of Muslims all over the world observe total abstention from food and water from dawn to sunset. Food and water is, however, permitted ad libitum between sunset and dawn (Husain *et al*. 1987; Toda & Morimoto, 2000). Hence, Ramadan fasting is in fact repeated cycles of fasting followed by refeeding every day and night for about 30 d.

Ramadan fasting in man results in increased serum lipids, uric acid, urea (Gumaa *et al*. 1978; Fedail *et al*. 1982; Hallack & Nomani, 1988; Nomani *et al*. 1989), NEFA and 3-OH butyrate, and a decrease in blood glucose, lactate and pyruvate (Malhotra *et al*. 1989; Nomani *et al*. 1989), indicating alterations in metabolic activities. The changes in urine volume, osmolarity, total solutes and ions (Na^+, K^+) and urea produce no adverse health effects on renal function (Cheah *et al.* 1990; Leiper *et al*. 2003). Basal metabolism slows down (Husain *et al.* 1987), whereas body fat is utilised efficiently during Ramadan fasting (El Ati *et al*. 1995). HDL cholesterol increases whereas LDL cholesterol decreases with Ramadan fasting (Adlouni *et al*. 1997; Benli Aksungar *et al*. 2005). Owing to different dietary habits and physical activities, both a gain (Frost & Pirani, 1987) and a loss (El Ati *et al*. 1995), and sometimes no change (Husain *et al*. 1987), in body weight have been reported after Ramadan fasting.

As millions of Muslims (young and old) have for centuries abstained from food and water in the daytime during the Islamic month of Ramadan, it seems important to examine the influence of this fasting schedule on human health, especially with respect to nutrition and energy metabolism. Although studying human biology is ideal, such studies are neither feasible nor ethical. Thus, the vast majority of current biomedical research is conducted using laboratory animals such as rats. In the present study, Ramadan-type fasting (RTF) was mimicked

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Abbreviations: BBM, brush border membrane; G6PDH, glucose-6-phosphate dehydrogenase; GGTase, γ-glutamyl transpeptidase; ICDH, isocitrate dehydrogenase; LAP, leucine aminopeptidase; LDH, lactate dehydrogenase; MDH, malate dehydrogenase; RTF, Ramadan-type fasting; SDH, succinate dehydrogenase.

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experimentally in rats used as a model for Ramadan fasting in man. Rats were fasted (12 h) and then refed (12 h) daily for 30 d, as practised by Muslims during the month of Ramadan. We hypothesised that RTF would result in specific intestinal and liver adaptations and alter metabolic activities.

To address this hypothesis, the influence of RTF on enzymes of carbohydrate metabolism and brush border membrane (BBM) in rat intestine and liver was determined. The activities of enzymes involved in glucose oxidation (e.g. lactate dehydrogenase (LDH), isocitrate dehydrogenase (ICDH), succinate dehydrogenase (SDH), malate dehydrogenase (MDH)) and its production (e.g. fructose 1,6-bisphosphatase, glucose-6-phosphatase) markedly increased in mucosal and liver homogenates in RTF compared with control rats. However, the enzymes of the BBM involved in the terminal digestion and/or absorption of nutrients decreased in intestine but increased in liver.

Materials and methods

Chemicals

Sucrose, p-nitrophenyl phosphate, sodium succinate, NADH and $NADP^+$ were purchased from Sigma Chemical Co. (St Louis, MO, USA). All other chemicals used were of analytical grade and were purchased from either Sigma Chemical Co. or Sisco Research Laboratory (Mumbai, India).

Experimental design

Unlike people, rats are nocturnal feeders, and it may be considered unphysiological to fast them in the daytime. It has, however, been reported that the rhythmic pattern of certain intestinal enzymes disappears in rats when they are fasted (for up to 5 d) on a daytime or night-time feeding schedule, and instead increases or changes either in anticipation of or in the presence of food (Saito *et al*. 1976). It has also been reported that a monosodium-glutamate-induced increase in alkaline phosphatase activity was not a consequence of actual day/night intake variations but due to a more general effect of monosodium glutamate characterised by neurohormonal and metabolic disturbances (Martinkova *et al*. 2000).

Considering the importance of Ramadan fasting, the effect of RTF was determined initially in rats that were fasted for 12 h followed by 12 h refeeding with either a daytime or a night-time feeding schedule for 30 d. The rats were killed at the end of last fast in the morning (day-fasters) or in the evening (night-fasters) after a stabilisation period of 10–12 h. It was noted that rats rushed to eat and drink immediately when food or water was given to them at the end of fasting period and then ate intermittently during the refeeding (12 h) time.

The results summarised in Table 1 shows that day/night RTF resulted in similar alterations in serum glucose, cholesterol, blood urea N, inorganic phosphate and LDH activity. As there was no significant difference between the respective controls, the values were pooled to make one control value. Various tissue enzymes also showed a similar pattern irrespective of day/night fasting–refeeding variations (see Results). It appeared that rats, irrespective of whether they were fasted by day or by night, showed adaptations similar to those observed earlier (Saito *et al*. 1976). Therefore a comprehensive effect of RTF was determined, as described later, by a daytime fasting (12 h) followed by a night-time refeeding (12 h) schedule; the results are compared with the nocturnal fasting schedule where appropriate.

Young adult Wistar rats weighing 135–155 g, fed with a standard pellet diet (Amrut Laboratories, Pune, India) and water ad libitum, were conditioned for 1 week before the start of the experiment. All animals were kept under conditions that prevented them from experiencing unnecessary pain and discomfort according to the guidelines approved by Ethical Committee. The rats were separated into two groups. One group was put on RTF (12 h fasting/12 h refeeding) for 30 d. The other group received their diet and water *ad libitum* both day and night and were used as a control. After 30 d, the rats were killed under light ether anaesthesia. The liver and entire small intestine, from the ligament of Trietz to the end of ileum, was removed. The intestines were washed by flushing them with ice-cold buffered saline (1 mmol/l Tris-HCl, 9 g/l NaCl, pH 7·4). The weights of the animals were recorded at the beginning and end of the experiment.

Preparation of homogenate

The washed intestines were slit in the middle, and the entire mucosa was gently scraped with a glass slide and weighed. A 15 g/l homogenate of this mucosa was prepared in ice-cold 100 mmol/l Tris-HCl, pH 7·4, using a Potter-Elvehejem homogeniser (Remi Motors, Mumbai, India) by passing five

Table 1. Effect of daytime and night-time Ramadan-type fasting on serum parameters
(Mean values with their standard errors and percentage change from control values (%) for 12 rats per group)

Group	Blood urea N (mg/dl)			Glucose (mg/dl)			Cholesterol (mg/dl)			Inorganic phosphorus (μmol/ml)			Alkaline phosphatase (units/dl)			Lactate dehydrogenase (units/ml)		
	Mean	SE	%	Mean	SE	%	Mean	SE	%	Mean	SE	%	Mean	SE	%	Mean	SE	%
Control	26·82	0·53		147·228	1·26		123·087	0·62		2·35	0·09		8·84	0·22		2·67	0·26	
Ramadan-type fasting																		
Day	26·09	0·47	−3	102·984	2·97*	−30	86·91	0·82*	−29	2·73	0·07*	+16	11·72	0·21*	+33	1·84	0·11*	−31
Night	26·85	0·88		110·84	2·58*	−25	78·85	0·52*	−36	3·03	0·02*	+29	9·17	0·07*	+4	1·82	0·27*	−32

*Mean values were significantly different from control at $P<0·05$ or higher degree of significance by group *t* test and ANOVA.

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pulses. The homogenate was centrifuged at 2000 ***g*** at 4 °C for 10 min to remove cell debris, and the supernatant thus obtained was used for assaying enzymes of carbohydrate metabolism. Liver homogenates were similarly prepared and analysed simultaneously. Aliquots of these homogenates were saved and kept at −20 °C until analysis.

Preparation of brush border membrane

BBM was prepared at 4 °C using differential precipitation by $CaCl_2$ (Kessler *et al*. 1978). Mucosa scraped from 4–5 washed intestines was used for each BBM preparation. This was homogenized in 50 mmol/l mannitol, 2 mmol/l Tris-HCl buffer, pH 7·5, in a glass homogeniser (Wheaton, IL, USA) with five complete strokes. The homogenate was then subjected to high-speed Ultra-Turrex Kunkel (Janke & Kunkel GmbH & Co. KG, Staufen, Germany) homogenation for three strokes of 15 s each with an interval of 15 s between each stroke. Solid $CaCl_2$ was added to the homogenate to a final concentration of 10 mmol/l, and the mixture stirred for 20 min on ice. The homogenate was centrifuged at 2000 ***g*** in a J2-21 Beckman centrifuge (J2 MI; Beckman Instruments, Palo Alto, CA, USA), and the supernatant was then recentrifuged at 352 000 ***g*** for 30 min. The pellet was resuspended in 50 mmol/l sodium maleate buffer, pH 6·8, with four passes by a loose-fitting Dounce homogeniser (Wheaton) in a 15 ml corex tube and centrifuged at 352 000 ***g*** for 20 min. The outer white fluffy pellet of BBM was resuspended in a small volume of sodium maleate buffer.

The membrane preparations were purified several magnitudes as the specific activities of the BBM enzymes were increased 7–10-fold compared with the homogenate. Aliquots of homogenates (after high-speed homogenisation) and BBM thus prepared were saved and stored at −20 °C until further analysis for the BBM enzymes sucrase, alkaline phosphatase and γ-glutamyl transpeptidase (GGTase).

Enzyme assays

The activities of marker enzymes in the homogenate and BBM fraction were determined by standard methods. The activity of alkaline phosphatase was measured by the method of Kempson *et al*. (1979) using p-nitrophenyl phosphate as a substrate, whereas sucrase was assayed by the method of Bernfeld (1955). GGTase was measured by the method of Glossmann & Neville (1972) and leucine aminopeptidase (LAP) by the method of Goldmann *et al.* (1976). The Michaelis Menton constant (K_m) and maximal velocity of the enzyme reaction (V_{max}) were determined by assaying these enzymes at various substrate concentrations (0·6–5·0 mmol/l for alkaline phosphatase, 5–160 mmol/l for sucrase, 0·1–0·4 mmol/l for LAP) and analysing the data by Lineweaver–Burk plot. Protein concentrations in BBM preparations and homogenates were determined by the method of Lowry *et al*. (1951) as modified by Yusufi *et al*. (1983).

The activities of LDH, MDH, glucose-6-phosphate dehydrogenase (G6PHD), malic enzyme and ICDH, involved in the oxidation of NADH or reduction of $NADP^+$, were determined by measuring extinction changes at 340 nm in a spectrophotometer (Cintra 5; GBC Scientific Equipment Pty, Victoria, Australia) using 3·0 ml assay mixture in a 1 cm cuvette at room temperature (28–30 °C). The net reaction rate was measured by the difference between the extinction values obtained prior to the addition of substrate and the values for the actual enzymic reaction following addition of the substrate. Appropriate blanks, in which the substrate was added after stopping the reaction, were run simultaneously.

All enzyme activities were measured under conditions in which enzyme reaction rates were linear with respect to incubation time and protein concentration using the method mentioned against each enzyme: LDH, E.C. 1·1·1·27 (Kornberg, 1955); MDH, E.C. 1·1·1·37 (Meyer *et al*. 1948); G6PDH, E.C. 1·1·1·49 (Shonk & Boxer, 1964); SDH, E.C. 1·3·99·1 (Szczepanska-Konkel *et al*. 1987); ICDH, E.C.1·1·1·42 (Ochoa, 1955*a*); malic enzyme, E.C. 1·1·1·40 (Ochoa, 1955*b*). Glucose-6-phosphatase E.C. 3·1·3·9 and fructose-1,6-bisphosphatase E.C 3·1·3·11 were assayed by the method of Shull *et al*. (1956). The inorganic phosphate liberated was measured by the method of Tausky & Shorr (1953).

Analysis of serum parameters

The serum samples were deproteinated with 3 % trichloroacetic acid in a ratio of 1:3 v/v. The samples were centrifuged at 2000 ***g*** (Remi centrifuge, India) for 10 min. The protein-free supernatant was used to estimate inorganic phosphate by the method of Tausky & Shorr (1953). Total serum cholesterol was estimated directly in serum samples by the method of Zlatkis *et al*. (1953). Urea was measured by the method of Fingerhut *et al*. (1966); glucose was estimated by o-toluidine method using kit from Span diagnostics (Surat, India).

Definition of unit

One unit of enzyme activity is the amount of enzyme required for the formation of 1 μmol product/h under specified experimental conditions. Specific activity is enzyme units/mg protein.

Statistical analysis

Results are expressed as means with their standard errors for at least three separate experiments. There were two groups of rats in each experiment: control and RTF group. Each sample of BBM and homogenate was prepared by pooling tissues from 4–5 rats. The data are representative of 12–15 rats per group per experiment. Where appropriate, statistical evaluation was conducted by group *t* test and ANOVA.

Results

The effect of RTF with 12 h daytime fasting and 12 h nighttime refeeding or vice versa for 30 d was studied in detail by assessing some serum parameters as well as the activities of certain enzymes from liver and small intestinal mucosa of rats that were involved in terminal digestion, absorption and carbohydrate metabolism.

https://doi.org/10.1017/BJN20061942 Published online by Cambridge University Press

Table 2. Effect of Ramadan-type fasting on body and mucosal weight of rats

(Mean values with their standard errors for three different experiments with four rats per group and change from control values (%))

Group	Body weight (g)			Mucosal weight (g/intestine)			Total mucosal protein (mg)			Mucosal weight:body weight ratio
	Mean	SE	%	Mean	SE	%	Mean	SE	%	
Control	245·83	7·67		4·3	0·47		1240·51	150		0·017
Ramadan-type fasting	226·92	8·66	−8	3·32	0·54	−23	989·38	98	−20	0·014

The initial mean body weight was 145·95 (SE 7·75)g for *n* 24 rats.

Effect of Ramadan-type fasting on serum parameters in daytime and night-time fasting conditions

As shown in Table 1, an effect of RTF was observed on various serum parameters during a daytime compared with night-time fasting schedule. Serum glucose, cholesterol and LDH activity significantly lowered, whereas inorganic phosphate increased under both the daytime and night-time fasting schedule. Blood urea N was not changed. The activity of serum alkaline phosphatase was, however, significantly increased with daytime fasting but only slightly increased with night-time fasting.

Effect of Ramadan-type fasting on body and mucosal weight of rats

The young adult rats used in the study showed a significant increase in body weight in both the control (+68 %) and the fasted (+55 %) rats compared with the weight recorded at the start of the experiment (145·97 (SE 7·75) g). The gain in body weight at the end of 30 d fasting period was slightly but not significantly lower in the RTF than the control rats. The mucosal weight was also lowered (−22 %; Table 2).

Effect of Ramadan-type fasting on brush border membrane enzymes in mucosal homogenates and isolated brush border membrane

RTF resulted in significant decrease in the activities of alkaline phosphatase, GGTase and sucrase in mucosal homogenates and in the isolated BBM preparations (Table 3). The enzyme activities similarly increased (7–10-fold) in the membrane preparations compared with respective values for the homogenate in both the control and RTF rats, indicating that the quality of membranes prepared by the procedure was similar for control and RTF rats. The specific activities of alkaline phosphatase, GGTase and sucrase all fell significantly (by approximately 25 %) in the homogenates. However, alkaline phosphatase activity decreased to greater extent (−38 %) than the activities of GGTase (−25 %) and sucrase (−20 %) in BBM preparations. In a preliminary experiment, it was observed that the activities of both alkaline phosphatase (−25 %) and sucrase (−20 %) declined similarly in daytime-fasted as well in night-time-fasted rats (data not shown). The kinetic parameters (K_m, V_{max}) of alkaline phosphatase and sucrase were also determined by assaying the enzymes in BBM preparations at different substrate concentrations. The results summarised in Table 4 show that the decrease in the activity of both alkaline phosphatase and sucrase caused by RTF was due mainly to a decrease in the V_{max} of the enzyme rather than to changed values of the Michaelis Menton constant (K_m).

Effect of Ramadan-type fasting on enzymes of carbohydrate metabolism in rat intestine

The specific activities of various enzymes involved in carbohydrate metabolism were determined in mucosal homogenates of control and RTF rats (daytime-fasted) after 30 d fasting. The activity of LDH, a representative of anaerobic glycolysis, markedly increased (+34 %) with RTF (LDH activity being similarly enhanced by night-time fasting). However, the

Table 3. Effect of Ramadan-type fasting on the activities of alkaline phosphatase, γ-glutamyl transpeptidase and sucrase in the mucosal homogenates and brush border membrane preparations

(Mean values with their standard errors for specific activities (μmol/mg protein per h) for three different experiments with their respective change from control values (%))

Group	Homogenate			Brush border membrane			× Enrichment
	Mean	SE	%	Mean	SE	%	
Alkaline phosphatase							
Control	7·46	0·05		56·48	0·72		7·6
Ramadan-type fasting	5·50	0·01*	−26	34·86	5·36*	−38	6·3
γ-Glutamyl transpeptidase							
Control	4·30	0·14*		38·50	0·55		9
Ramadan-type fasting	3·25	0·02*	−24	28·75	1·68*	−25	8·8
Sucrase							
Control	55·20	0·09		540·63	12·5		9·7
Ramadan-type fasting	42·00	1·64*	−24	433·80	5·70*	−20	10·32

* Means were significantly different from control at $P < 0.05$ or higher degree of significance by group *t* test and ANOVA.

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Table 4. Effect of Ramadan fasting on the kinetic parameters of alkaline phosphatase and sucrase

(Means values with their standard errors for three different experiments with their respective change from control values (%))

	K_m ($\times10^{-3}$ moL/L)		V_{max} (μmol/mg protein per h)		
Group	Mean	SE	Mean	SE	%
Alkaline phosphatase					
Control	0·90	0·04	41·66	3·27	
Ramadan-type fasting	0·90	0·01	32·25	1·27*	−23
Sucrase					
Control	41·6	0·01	333	30·24	
Ramadan-type fasting	41·6	0·06	222	18·32*	−33

K_m (Michaelis Menton constant) and V_{max} (maximal velocity of enzyme reaction) were determined in brush border membrane preparations.
*Means were significantly different from control at $P<0·05$ or higher degree of significance by group t test and ANOVA.

activities of ICDH, SDH and MDH, enzymes of glucose oxidation, profoundly increased after the 0 d RTF period. The activity of ICDH increased significantly (+63 %), whereas the activities of SDH (+83 %) and MDH (+106 %) increased to much greater extent compared with control values (Table 5).

The effect of RTF on the activities of gluconeogenic enzymes fructose 1,6-bisphosphatase and glucose-6-phosphatase was also determined. The activities of these enzymes were also enhanced during RTF compared with values in the control rats, although the increase was smaller compared with that seen with the enzymes involved in glycolysis (LDH) and the tricarboxylic acid cycle (ICDH, SDH, MDH). The activities of G6PDH (hexose monophosphate shunt) and malic enzyme, which play important role in reducing anabolic pathways by supplying NADPH, were also studied. In contrast to the enzymes of glucose oxidation and production, the activities of both G6PDH (−33 %) and malic enzyme (−36 %) significantly declined in rat mucosa after RTF (Table 6).

Effect of Ramadan-type fasting on enzymes of carbohydrate metabolism in rat liver homogenates

The effect of RTF in both a daytime and night-time fasting schedule are shown in Table 7. The specific activities of LDH and MDH, enzymes involved in glucose degradation, profoundly increased in both fasting schedules. The activities of gluconeogenic enzymes glucose-6-phosphatase and fructose 1,6-bisphosphatase also markedly enhanced after RTF in the liver homogenates irrespective of day/night variations in the feeding schedule. The effect was more prominent in the liver than in intestinal enzymes. In contrast to intestine, where the activities of G6PDH and malic enzyme declined, the activities of these enzymes profoundly increased to a similar extent in both fasting conditions. Compared with intestinal enzymes, the activities of alkaline phosphatase, GGTase and LAP were significantly increased in the liver homogenates (Table 8) from both daytime-fasted and night-time-fasted RTF rats. Kinetic analysis showed that the increase in LAP activity (+71 %) was mainly due to an alteration in the V_{max} of LAP activity (RTF: 3·33 (SE 0·08) *v*. control: 1·96 (SE 0·02), whereas K_m was unchanged (data not shown).

Discussion

The main purpose of the present study was to determine the influence of RTF on certain enzymes involved in carbohydrate metabolism and terminal digestion in the intestine and liver of rats used as a model of human Ramadan fasting. The present results in part support our hypothesis that RTF results in specific intestinal and liver metabolic adaptations. Indeed,

Table 5. Effect of Ramadan type fasting on the specific activities of lactate dehydrogenase (LDH), isocitrate dehydrogenase (ICDH), succinate dehydrogenase (SDH) and malate dehydrogenase (MDH) in homogenates of intestinal mucosa

(Mean values with their standard errors for specific activities (μmol/mg protein per h) for three different experiments with their respective change from control values (%))

	LDH			ICDH			SDH			MDH		
Group	Mean	SE	%	Mean	SE	%	Mean	SE	%	Mean	SE	%
Control	89·75	6·35		0·140	0·009		0·258	0·04		85·27	5·35	
Ramadan-type fasting	120	5·26*	+34	0·228	0·013*	+63	0·471	0·03*	+83	175·71	10·31*	+106

*Means were significantly different from control at $P<0·05$ or higher degree of significance by group t test and ANOVA.

Table 6. Effect of Ramadan-type fasting on the specific activities of fructose 1,6-bisphosphatase, glucose-6-phosphatase (G6Pase), glucose-6-phosphate dehydrogenase (G6PDH) and malic enzyme in homogenates of intestinal mucosa

(Mean values with their standard errors for specific activities (μmol/mg protein per h) for three different experiments with their respective change from control values (%))

	Fructose 1,6-bisphosphatase			G6Pase			G6PDH			Malic enzyme		
Group	Mean	SE	%	Mean	SE	%	Mean	SE	%	Mean	SE	%
Control	0·47	0·01		0·98	0·01		0·323	0·011		0·473	0·017	
Ramadan-type fasting	0·57	0·02*	+21	1·15	0·04*	+17	0·216	0·012*	−33	0·305	0·036*	−36

*Means were significantly different from control at $P<0·05$ or higher degree of significance by group t test and ANOVA.

Table 7. Effect of daytime and night-time Ramadan-type fasting on the specific activities of lactate dehydrogenase (LDH), malate dehydrogenase (MDH), fructose 1,6-bisphosphatase (FBPase), glucose-6-phosphatase (G6Pase), gluose-6-phosphate dehydrogenase (G6PDH) and malic enzyme in liver homogenate

(Mean values with their standard errors for specific activities (μmol/mg protein per h) for three different experiments with their respective change from control values (%))

Group	LDH			MDH			FBPase			G6Pase			G6PDH			Malic enzyme		
	Mean	SE	%	Mean	SE	%	Mean	SE	%	Mean	SE	%	Mean	SE	%	Mean	SE	%
Control	8·94	0·49		8·54	0·48		0·75	0·05		0·16	0·01		1·29	0·14		1·23	0·10	
Ramadan-type fasting																		
Day	14·89	0·39*	+67	13·76	0·32*	+61	1·02	0·05*	+35	0·26	0·02*	+62	2·61	0·20*	+102	2·26	0·29*	+83
Night	14·36	0·27*	+61	13·00	0·14*	+52	1·06	0·05	+41	0·26	0·01*	+63	2·61	0·20*	+102	2·21	0·10*	+79

* Means were significantly different from control at $P < 0.05$ or higher degree of significance by group *t* test and ANOVA.

the activities of enzymes belonging to various metabolic pathways, for example glycolysis, the tricarboxylic acid cycle and gluconeogenesis, profoundly increased in both the intestine and liver with RTF.

In general, a 30 d 12 h fasting/12 h feeding schedule was associated with a steady gain in body weight in both control (+68 %) and fasted (+55 %) young adult rats compared with their starting weights (Table 2). At the end of 30 d period, however, body weight was slightly (−8 %) but insignificantly lower in RTF compared with control rats, as has previously been observed in some studies on people (Hallack & Nomani, 1988; Adlouni *et al*. 1997, 1998; Maislos *et al*. 1998; Yucel *et al*. 2004). The loss of body weight in human subjects has been attributed to either dehydration or loss of body fat during the course of fasting (Roky *et al*. 2004). The lowering of serum glucose, cholesterol and LDH activity in rats is in partial agreement with the reported decrease in blood glucose, lactate and pyruvate in people after Ramadan fasting (Fedail *et al*. 1982; Hallack & Nomani, 1988; Malhotra *et al*. 1989).

The results of the present study demonstrate that the enzymes involved in glucose degradation and production were significantly enhanced in both the mucosa and the liver (Tables 5–7). Anaerobic glycolysis, compared with oxidative metabolism, has been shown to be the major source of energy in the rat intestine (Hubscher & Sherrat, 1962). The marked increase in ICDH, SDH and MDH together with LDH activity in the present study, however, suggests that oxidative metabolism in addition to glycolysis becomes involved in increased energy production during prolonged intermittent RTF. Increased fatty acid oxidation (El Ati *et al.* 1995) may result in enhanced activities of enzymes in the tricarboxylic acid cycle. The activities of G6PDH and malic enzyme, which act to produce NADPH, were differentially altered by RTF. Although the activities of these enzymes significantly declined in the intestine, they were profoundly increased in the liver. Increased NADPH thus produced at least in the liver may have supported many reducing anabolic reactions needed during prolonged intermittent fasting.

The underlying mechanism by which RTF may cause alterations in liver and intestinal metabolic activities seems the composite effects of fasting and refeeding. Fasting is known to produce extensive morphological as well as biochemical changes in the small intestine and liver (Dou *et al*. 2001; Martins *et al*. 2001); these include decreased mucosal weight, a reduction in the number and length of microvilli and hence a lowering of the total surface area of the villi and microvilli (Geyra *et al*. 2001). Refeeding, in contrast, causes a reversal of these effects (Butzner & Gall, 1990).

Furthermore, the activities of certain enzymes involved in carbohydrate metabolism decrease after fasting (Anderson & Zakim, 1970; Budhoski *et al*. 1982; Farooq *et al*. 2004) and are restored upon refeeding (Stifel *et al*. 1968, 1969; Shakespeare *et al*. 1969; Kotler *et al*. 1982). In contrast, gluconeogenesis is known to increase with fasting and decrease upon refeeding. A rapid degradation of various proteins and enzymes has also been reported by fasting, whereas refeeding results in increased protein/enzyme synthesis (Holt & Yeh, 1992; Boza *et al*. 1999). The profound increase in the activities of the enzymes of glycolysis, of the tricarboxylic acid cycle and to some extent of gluconeogenesis by RTF might be due to an enhanced synthesis of these enzymes resulting from repeated 12 h fasting/12 h

https://doi.org/10.1017/BJN20061942 Published online by Cambridge University Press

https://doi.org/10.1017/BJN20061942 Published online by Cambridge University Press

Table 8. Effect of daytime and night-time Ramadan-type fasting on the specific activities of alkaline phosphatase, γ-glutamyl transpeptidase and leucine aminopeptidase in liver homogenate

(Mean values with their standard errors for specific activities (μmol/mg protein per h) for three different experiments with their respective change from control values (%))

	Alkaline phosphatase			γ-Glutamyl transpeptidase			Leucine aminopeptidase		
Group	Mean	SE	%	Mean	SE	%	Mean	SE	%
Control	1·94	0·25		1·37	0·03		2·81	0·14	
Ramadan-type fasting									
Day	2·70	0·27*	+ 39	4·31	0·21*	+ 215	4·87	0·14*	+ 73
Night	3·05	0·31*	+ 57	3·52	0·08*	+ 157	4·63	0·10*	+ 64

* Means were significantly different from control at $P < 0.05$ or higher degree of significance by group *t* test and ANOVA.

refeeding for 30 d. The respective increase or decrease in the activities of alkaline phosphatase, GGTase, LAP and/or sucrase in liver and intestine caused by RTF was found to be due to alterations in the V_{max} rather than K_m values. These observations also indicate adaptive but specific alterations in protein/enzyme synthesis. The other regulatory mechanisms might be activated by repeated cycles of a 30 d fasting/refeeding schedule. Elevated serum thyroid hormone levels, as observed in human subjects during Ramadan fasting, might be one of such factors responsible for enhanced metabolic activity (Fedail *et al*. 1982).

It has been reported that short-term fasting followed by refeeding gave rise to a disappearance of circadian activity and that the alterations observed were actually produced in anticipation of food, rather than in its presence, by specific adaptative mechanisms (Saito *et al*. 1976) similar to the learning reflexes put forward long ago by Pavlov. It seems reasonable to suggest that rats can be used as a model of human Ramadan fasting because of the similar alterations observed in some blood parameters in rats and reported in man, and also because of similar metabolic changes observed in the daytime and night-time fasting schedules.

We therefore conclude that RTF in rats results in specific adaptive changes in the metabolic activities of both the intestine and the liver. The increased activities of enzymes involved in the degradation as well as the production of glucose suggest that RTF enhances nutrition and energy metabolism. The results provide useful information of significant clinical importance on adaptations to unusual eating habits with restricted energy intake.

Acknowledgements

The Council of Scientific and Industrial Research, New Delhi, India, is acknowledged for its award of a Research Associate fellowship to N. F. Financial support to the department from the University Grant Commission and the Department of Science and Technology, and a research grant (SO/SO/B-93/89) from the Department of Science and Technology to A. N. K. Y. is also gratefully acknowledged.

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https://doi.org/10.1017/BJN20061942 Published online by Cambridge University Press