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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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 abstinence 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. 1979; 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, osmolality, 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.

Abbreviations: BBM, brush border membrane; G6PDH, glucose-6-phosphate dehydrogenase; GGTag, 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 refeding 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 refeding (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 refeding (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-Elvehejhem 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)

<table>
<thead>
<tr>
<th>Group</th>
<th>Blood urea N (mg/dl)</th>
<th>Glucose (mg/dl)</th>
<th>Cholesterol (mg/dl)</th>
<th>Inorganic phosphorus (µmol/ml)</th>
<th>Alkaline phosphatase (units/dl)</th>
<th>Lactate dehydrogenase (units/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Day</td>
<td>26·82  ± 0·53</td>
<td>147·228 ± 1·26</td>
<td>123·087 ± 0·62</td>
<td>2·35 ± 0·09</td>
<td>8·84 ± 0·22</td>
<td>2·67 ± 0·26</td>
</tr>
<tr>
<td>Night</td>
<td>26·09  ± 0·47</td>
<td>102·984 ± 2·97*</td>
<td>86·91 ± 0·82* − 30</td>
<td>2·73 ± 0·07* + 16</td>
<td>11·72 ± 0·21* + 33</td>
<td>1·84 ± 0·11* − 31</td>
</tr>
</tbody>
</table>

*Mean values were significantly different from control at *P* < 0·05 or higher degree of significance by group f test and ANOVA.
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
CaCl₂ (Kessler et al. 1978). Muscosa 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 sub-
jected to high-speed Ultra-Turrex Kunkel (Janke & Kunkel
Gmb & Co. KG, Staufen, Germany) homogenation for three
strokes of 15 s each with an interval of 15 s between
each stroke. Solid CaCl₂ 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 lnstruments,
Palo Alto, CA, USA), and the supernatant was then recentri-
gufed 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 fluffly pellet of BBM was resuspended in a small
volume of sodium maleate buffer.

The membrane preparations were purified several magni-
tudes 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 Kemp-
sen et al. (1979) using p-nitrophenyl phosphate as a substrate,
whereas sucrase was assayed by the method of Bernfeld
et al. (1955). GGTase was measured by the method of Glossmann
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 phospha-
tase, 5–160 mmol/l for sucrase, 0·1–0·4 mmol/l for LAP) and
analysing the data by Lineweaver–Burk plot. Protein concen-
trations in BBM preparations and homogenates were deter-
mined by the method of Lowry et al. (1951) as modified by
Yusufi et al. (1983).

The activities of LDH, MDH, glucose-6-phosphate dehy-
drogenase (G6PDH), malic enzyme and ICDH, involved in
the oxidation of NADH or reduction of NADP⁺, were deter-
mined by measuring extinction changes at 340 nm in a spec-
trophotometer (Cnitra 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 incu-
bation time and protein concentration using the method men-
tioned 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, 1955α); malic enzyme, E.C. 1·1·1·40 (Ochoa, 1955β);
Glucose-6-phosphatase E.C. 3·1·3·9 and fructose-1,6-bisphos-
phatase 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 % trichloro-
acetic 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 exper-
imental 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 tis-
sues from 4–5 rats. The data are representative of 12–15 rats
per group per experiment. Where appropriate, statistical eval-
uation was conducted by group t test and ANOVA.

Results

The effect of RTF with 12 h daytime fasting and 12 h night-
time 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.
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 nighttime fasting schedule. Serum glucose, cholesterol and LDH activity significantly lowered, whereas inorganic phosphate increased under both the daytime and nighttime 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 nighttime 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<sub>m</sub>, V<sub>max</sub>) 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<sub>max</sub> of the enzyme rather than to changed values of the Michaelis Menton constant (K<sub>m</sub>).

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>
<thead>
<tr>
<th>Group</th>
<th>Mean (SE)</th>
<th>%</th>
<th>Mean (SE)</th>
<th>%</th>
<th>Mean (SE)</th>
<th>%</th>
<th>Mucosal weight/body weight ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>Body weight (g)</td>
<td></td>
<td></td>
<td>Mucosal weight (g/intestine)</td>
<td></td>
<td>Total mucosal protein (mg)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>245.83 (7.67)</td>
<td>-8</td>
<td>4.3 (0.47)</td>
<td>-26</td>
<td>1240.51 (150)</td>
<td>-20</td>
<td>0.017</td>
</tr>
<tr>
<td>Ramadan-type fasting</td>
<td>226.92 (8.66)</td>
<td>-8</td>
<td>3.32 (0.54)</td>
<td>-23</td>
<td>989.38 (98)</td>
<td>-20</td>
<td>0.014</td>
</tr>
</tbody>
</table>

The initial mean body weight was 145.95 (se 7.75) g for n 24 rats.

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

<table>
<thead>
<tr>
<th>Group</th>
<th>Mean (SE)</th>
<th>%</th>
<th>Mean (SE)</th>
<th>%</th>
<th>Mean (SE)</th>
<th>%</th>
<th>x Enrichment</th>
</tr>
</thead>
<tbody>
<tr>
<td>Homogenate</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Alkaline phosphatase</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>7.46 (0.05)</td>
<td>-26</td>
<td>56.48 (0.72)</td>
<td>-26</td>
<td>7.6</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ramadan-type fasting</td>
<td>5.50 (0.01*)</td>
<td>-26</td>
<td>34.86 (5.36*)</td>
<td>-26</td>
<td>6.3</td>
<td></td>
<td></td>
</tr>
<tr>
<td>γ-Glutamyl transpeptidase</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>4.9 (0.14*)</td>
<td>-24</td>
<td>38.50 (0.55)</td>
<td>-24</td>
<td>9</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ramadan-type fasting</td>
<td>3.25 (0.02*)</td>
<td>-24</td>
<td>28.75 (1.68*)</td>
<td>-24</td>
<td>8.8</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sucrase</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>55.20 (0.09)</td>
<td>-24</td>
<td>540.63 (12.5)</td>
<td>-24</td>
<td>9.7</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ramadan-type fasting</td>
<td>42.00 (1.64*)</td>
<td>-24</td>
<td>433.80 (5.70*)</td>
<td>-24</td>
<td>10.32</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*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 observed in rats used as a model of human Ramadan fasting. The present results in part support our hypothesis that RTF results in profound metabolic changes and are consistent with previous reports indicating that 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 irrespective of day/night variations in the feeding schedule. The effect was more prominent in the liver than in intestinal enzymes.

**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 (%))

<table>
<thead>
<tr>
<th>Group</th>
<th>$K_m$ (x10$^{-2}$ mol/L)</th>
<th>$V_{max}$ (µmol/mg protein per h)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>0.90 ± 0.01</td>
<td>41.66 ± 3.27</td>
</tr>
<tr>
<td>Ramadan-type fasting</td>
<td>0.90 ± 0.01</td>
<td>32.25 ± 1.27*</td>
</tr>
<tr>
<td>Sucrase</td>
<td>41.6 ± 0.01</td>
<td>333 ± 30.24</td>
</tr>
<tr>
<td>Ramadan-type fasting</td>
<td>41.6 ± 0.06</td>
<td>222 ± 18.32*</td>
</tr>
</tbody>
</table>

*$K_m$ (Michaelis Menton constant) and $V_{max}$ (maximal velocity of enzyme reaction) were determined in brush border membrane preparations.

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.

**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**

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

<table>
<thead>
<tr>
<th>Group</th>
<th>LDH</th>
<th>ICDH</th>
<th>SDH</th>
<th>MDH</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>89.75 ± 0.36</td>
<td>0.140 ± 0.009</td>
<td>0.258 ± 0.04</td>
<td>85.27 ± 5.35</td>
</tr>
<tr>
<td>Ramadan-type fasting</td>
<td>120 ± 5.26*</td>
<td>0.228 ± 0.013*</td>
<td>0.471 ± 0.03*</td>
<td>175.71 ± 10.31*</td>
</tr>
</tbody>
</table>

*Means were significantly different from control at $P < 0.05$ 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**

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

<table>
<thead>
<tr>
<th>Group</th>
<th>Fructose 1,6-bisphosphatase</th>
<th>G6Pase</th>
<th>G6PDH</th>
<th>Malic enzyme</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>0.47 ± 0.01*</td>
<td>0.98 ± 0.01</td>
<td>0.323 ± 0.011</td>
<td>0.473 ± 0.017</td>
</tr>
<tr>
<td>Ramadan-type fasting</td>
<td>0.57 ± 0.02*</td>
<td>1.15 ± 0.04*</td>
<td>0.216 ± 0.012*</td>
<td>0.305 ± 0.036*</td>
</tr>
</tbody>
</table>

*Means were significantly different from control at $P < 0.05$ 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), glucose-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 (%)).

<table>
<thead>
<tr>
<th>Group</th>
<th>LDH Mean SE</th>
<th>% Change</th>
<th>MDH Mean SE</th>
<th>% Change</th>
<th>FBPase Mean SE</th>
<th>% Change</th>
<th>G6Pase Mean SE</th>
<th>% Change</th>
<th>G6PDH Mean SE</th>
<th>% Change</th>
<th>Malic enzyme Mean SE</th>
<th>% Change</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>8.94 0.49</td>
<td>0.00</td>
<td>13.76 0.42</td>
<td>0.00</td>
<td>1.06 0.05</td>
<td>0.00</td>
<td>2.60 0.10</td>
<td>0.00</td>
<td>2.10 0.03</td>
<td>0.00</td>
<td>1.25 0.08</td>
<td>0.00</td>
</tr>
<tr>
<td>Ramadan-type fasting Day</td>
<td>14.89 0.27</td>
<td>102.38</td>
<td>13.76 0.14</td>
<td>92.57</td>
<td>2.61 0.02</td>
<td>92.57</td>
<td>2.61 0.02</td>
<td>92.57</td>
<td>2.61 0.02</td>
<td>92.57</td>
<td>2.61 0.02</td>
<td>92.57</td>
</tr>
<tr>
<td>Ramadan-type fasting Night</td>
<td>14.36 0.27</td>
<td>102.38</td>
<td>13.00 0.14</td>
<td>92.57</td>
<td>2.61 0.02</td>
<td>92.57</td>
<td>2.61 0.02</td>
<td>92.57</td>
<td>2.61 0.02</td>
<td>92.57</td>
<td>2.61 0.02</td>
<td>92.57</td>
</tr>
</tbody>
</table>

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

<table>
<thead>
<tr>
<th>Group</th>
<th>Alkaline phosphatase</th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean SE %</td>
<td>Mean SE %</td>
<td>Mean SE %</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>1.94 0.25</td>
<td>1.37 0.03</td>
<td>2.81 0.14</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ramadan-type fasting</td>
<td>Day 2.70 0.27* +39</td>
<td>4.31 0.21* +215</td>
<td>4.87 0.14* +73</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Night 3.05 0.31* +57</td>
<td>3.52 0.08* +157</td>
<td>4.63 0.10* +64</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* 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 succrase 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 adaptive 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.

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References


