Hormone-substrate responses to total fasting in lean and obese mice

GUY S. CUENDET, ERNEST G. LOTEN, DONALD P. CAMERON, ALBERT E. RENOLD, AND ERROL B. MARLISS
Institut de Biochimie Clinique, Département de Médecine, Université de Genève, Geneva, Switzerland

The hormone-substrate responses to total fasting in lean and obese mice. Am. J. Physiol. 228(1): 276–283. 1975.—The hormone-substrate milieu has been investigated in male fasted lean (C57BL/6+/-) mice and mutant obese mice of the same strain (C57BL/6-obob). The lean mouse, in winter, mobilized insufficient fat (due to inadequate stores) to permit survival beyond 3 days and was unable to achieve any degree of conservation of vital protein stores. By contrast, in summer, the same animals survived 7 days and showed evidence of greater and more sustained fat mobilization and ketosis and the ability to conserve protein. The insulin, glucagon, and insulin/glucagon molar ratios changed in both groups in a direction consistent with conversion to a catabolic state, and hence were probably largely responsible for the mobilization of substrates and stimulation of gluconeogenesis and ketogenesis. The seasonal difference in response is unexplained. The obob mouse, generally employed as a model for obesity, hyperglycemia, and hyperinsulinemia showed these features but also adapted to fasting in a fashion largely analogous to that of man renders it a useful model for the study of metabolism in this state, with the potential of applicability of findings to man.

prolonged fasting; intermediary metabolism; glucose; gluconeogenesis; ketone bodies; ketosis; insulin; glucagon; insulin/glucagon ratio; obesity; obob mice; endocrine pancreas; protein sparing

The human studies thus far performed have shown that the fasting adaptation consists in altered fuel mobilization and consumption so as to selectively utilize fat, the most efficient energy storage form. This allows for conservation of body protein, which exists in minimal quantities by contrast to fat (in terms of calories available when mobilized), and the loss of greater than half of which is regarded as incompatible with survival. This protein sparing is the result of an adaptation of most tissues, including the central nervous system, to the oxidation of either fatty acids or ketone bodies as principal fuel. In particular, the mechanisms whereby progressive curtailment of amino acid mobilization from protein stores (usually cited as primarily muscle protein) is achieved remains at present hypothetical. The origin of the carbon skeletons which are released from muscle principally as alanine (4) and glutamine (5) remains the subject of controversy (6, 8). Similarly, the mechanism whereby the central nervous system adapts to ketone body oxidation rather than glucose oxidation, remains incompletely defined (20, 31, 35).

Such considerations have led to the pursuit of an appropriate animal model which demonstrates these adaptations and in which the intimate mechanisms might be defined. Studies of other fasted laboratory rodents have, in general, demonstrated quite different responses from those observed in man (3, 4). Such animals have been unable to survive prolonged fasting periods because of lack of adequate adipose tissue stores. The present studies demonstrate that the same occurs in the lean mouse (C57BL), though with a marked seasonal variation. By contrast, the obese mutant of the same genetic strain (the C57BL-obob) is able to achieve prolonged survival of fasting. This obese mouse, generally studied in the context of a model for obesity, hyperglycemia, and hyperinsulinemia, thus provides a model which is acceptable to further study of the adaptive mechanisms referred to, although some may differ from those found in man.

METHODS AND MATERIAL

Experimental procedure. C57BL+/- lean mice and obese mice of the same strain homozygous for the ob gene (C57BL/6-obob) were obtained from the Jackson Laboratories (Bar Harbor, Maine). The lean mice were expressly not littermates of the obese, since it was considered preferable to exclude mice heterozygous for the ob gene from the controls. These mice are now recognized to give a phenotype in some
RESPONSES OF LEAN AND OBESE MICE TO FASTING

RESPECTS INTERMEDIATE BETWEEN THE HOMOZYGOUS CONDITIONS. ALL ANIMALS STUDIED WERE MALES.

BOTH GROUPS RECEIVED LABORATORY CHOW AD LIBITUM FOR 3 WK PRIOR TO THEIR USE.1 THEY WERE KEPT IN AN ENVIRONMENT IN WHICH TEMPERATURE WAS KEPT CONSTANT AT 25°C AND HUMIDITY AT 65%. ALL LIGHTING WAS ARTIFICIAL AND THE ANIMAL ROOM WAS ILLUMINATED FROM 0700 TO 1900 H DAILY, IRRESPECTIVE OF SEASON.

MICE OF EQUIVALENT AGE (8 WK) WERE EMPLOYED. HENCE, THE INITIAL WEIGHTS WERE GREATER FOR THE OBESE THAN FOR THE LEAN MICE. FOR URINE COLLECTIONS, MICE WERE HOUSED IN INDIVIDUAL, SPECIALLY CONSTRUCTED METABOLIC CAGES. WATER WAS PERMITTED AD LIBITUM, BUT NO FOOD, VITAMINS, OR SALTS WERE GIVEN. THE SAME MICE WERE KEPT FOR THE ENTIRE DURATION OF THE FAST IN THESE CAGES. AT 24 H INTERVALS THE URINE (COLLECTED WITH THYMOL CRYSTALS TO INHIBIT BACTERIAL CONTAMINATION) WAS REMOVED AND THE SILICONIZED COLLECTING FUNNELS WERE RINSED WITH DOUBLY DISTILLED WATER TO GIVE A FINAL VOLUME OF 15 ML, REPRESENTING THE COLLECTION FOR THAT PERIOD, WHICH WAS IMMEDIATELY FROZEN AND MAINTAINED AT -20°C UNTIL ANALYSIS. VALUES FOR URINE REPRESENT THE SAME GROUP OF ANIMALS STUDIED OVER THE WHOLE EXPERIMENTAL PERIOD. (IT WAS CONSIDERED THAT THE TECHNIQUE OF URINE COLLECTION PRECLUDED USE OF THESE URINE SAMPLES FOR DETERMINATION OF UNSTABLE SUBSTANCES, INCLUDING AMMONIA AND KETONE BODIES.)

URINE AND BLOOD COLLECTIONS WERE MADE FROM A ZERO TIME REFERENCE POINT REPRESENTING THE "FED" STATE, AT 0900 H WITH FREE ACCESS TO FOOD UP TO THIS TIME. AN ADEQUATE VOLUME OF BLOOD NECESSITATED EXSANGLUINATION OF THE ANIMALS AND, HENCE, DIFFERENT TIME PERIODS ARE REPRESENTED BY DIFFERENT ANIMALS. BLOOD SAMPLING WAS AT 0, 16 H, AND AT 2, 8, AND 16 DAYS IN THE OBESE, AND FOR THE LEAN MICE, AT 0, 1, 2, AND 3 DAYS. HOWEVER, A DIFFERENCE IN THE LENGTH OF SURVIVAL OF THE LEAN MICE OCCURRED FROM WINTER TO SUMMER, WITH CORRESPONDING ALTERATIONS IN THE MEASURED PARAMETERS. HENCE, A FURTHER STUDY WAS PERFORMED IN THE LEAN MICE, PROLONGED TO 7 DAYS DURING THE SUMMER. BLOOD WAS COLLECTED AFTER DECAPITATION, WITHOUT PRIOR ANESTHESIA. TOTAL TIME FROM DECAPITATION TO COMPLETION OF COLLECTION WAS 30-60 S. THE BLOOD WAS COLLECTED IN HEPARINIZED MICROTUBES CONTAINING 1,000 KIU TRASYLOL (BAYER PHARMA, A.G., ZUERICH, SWITZERLAND) PER MILLILITER OF BLOOD, AND AN ALIQUOT WAS IMMEDIATELY DEPROTEINIZED IN AN EQUAL VOLUME OF COLD 30% (WT/VOL) PERCHLORIC ACID. THE REMAINDER WAS CENTRIFUGED AND AN ALIQUOT OF PLASMA WAS DEPROTEINIZED IN AN EQUAL VOLUME OF COLD (10% WT/VOL) PERCHLORIC ACID. ALL FILTRATES AND REMAINING PLASMA SAMPLES WERE IMMEDIATELY STORED AT -20°C, WHERE THEY WERE KEPT UNTIL ANALYSIS. AFTER BLOOD WAS COLLECTED, PART OF THE LIVER WAS RAPIDLY REMOVED AND PLACED IN LIQUID NITROGEN AND SUBSEQUENTLY STORED AT -20°C UNTIL ANALYSIS FOR GLYCOGEN IN THE LEAN MICE. THE PANCREAS WAS THEN REMOVED, FROZEN, AND SUBSEQUENTLY EXTRACTED IN ACID-ETHANOL AFTER DISRUPTION OF THE CELLS BY SONICATION WITH A B-12 SONIFIER (BRANSON SONIC POWER COMPANY, DANBURY, CONN.). A SMALL NUMBER OF CARCASSES FROM LEAN ANIMALS WERE HOMOGENIZED IN METHANOL AND EXTRACTED IN CHLOROFORM, AND THE EXTRACT WAS DRIED AND WEIGHED AS AN INDEX OF TOTAL-BODY LIPIDS.

BIOCHEMICAL METHODS. URINE CREATININE WAS DETERMINED BY AN ALKALINE PICRATE METHOD AND UREA WAS DETERMINED BY THE UREA METHOD. PLASMA GLUCOSE WAS ESTIMATED WITH A GLUCOSE

1 THE CHOW WAS THE SAME FOR Lean AND Obese, AND ITS COMPOSITION WAS UNALTERED IN THE SUMMER Versus WINTER STUDIES.

Oxidase method using an automatic recording spectrophotometer (LKB Produkter AB, Stockholm-Brommer, Sweden). Blood perchloric acid filtrates were used for determination of lactate (32), pyruvate (33), beta-hydroxybutyrate (39), glutamine (26), and glutamate (5). These determinations were performed using an Aminco Fluorometer (American Instrument Co., Inc., Silver Spring, Md.) with automatic sample changer and recorder. Glutamine, pyruvate, and acetoacetate assays were performed within 2 days of sampling. Under the conditions employed, no significant decrease in concentration of these substances could be demonstrated, provided that freezing was immediate and the sample was thawed only once, just prior to assay. The enzymic fluorimetric methods cited were modified to enable determination on as little as 10 μL of unneutralized filtrate, by decreasing the amount of nucleotide cofactors to levels appropriate to the quantity of substrate in the samples. With the exception of glutaminase (Worthington Biochemical Corp., Freehold, N. J.), all enzymes and cofactors were obtained from C. F. Boehringer GmbH, Mannheim, W Germany (through the courtesy of Dr. Felix Schmidt). Substrates were obtained from E. Merck, A.G., Darmstadt, W Germany. Plasma free fatty acids (FFA) were estimated by the radiochemical microtechnique of Ho (22).

Liver glycogen was estimated by the glucose oxidase method, applied to samples after dissolution of liver in hot KOH, subsequent precipitation with cold ethanol, and acid hydrolysis of the purified glycogen.

Hormone assays. Plasma insulin concentrations (IRI) were estimated using a double-antibody radioimmunoassay system miniaturized as previously described (11) and employing an antiinsulin serum reacting poorly with proinsulin, and purified mouse insulin as standard (24 U/mg, supplied by Novo Research Institute, Copenhagen). Plasma immunoreactive glucagon (IRG) was determined with an antibody relatively specific for pancreatic glucagon (30K, supplied by Dr. R. H. Unger) in an assay system described previously (38), using porcine glucagon standard. The assay for glucagon is considered to provide values representing immunoreactivity originating primarily in the pancreas. This is based upon data demonstrating the poor cross-reactivity of the antiserum employed, with gut extracts. However, cross-reactivity with pancreatic glucagon fragments or a prohormone in plasma is not excluded. For both hormones, dilution curves of the pancreatic extracts were superimposable upon the standard curves. Pancreatic immunoreactive glucagon was assayed as was plasma IRG, while pancreatic IRI was assayed as described previously (21), using a mouse insulin standard. Statistical analyses were performed according to Snedecor (36) utilizing the unpaired Student t test.

RESULTS

THE PERIOD OF FASTING IN EACH OF THESE GROUPS OF MICE WAS DETERMINED IN PRELIMINARY OBSERVATIONS OF THE DURATION OF SURVIVAL. FOR THE LEAN MICE, SURVIVAL IN WINTER (NOVEMBER AND JANUARY) WAS LIMITED TO 3.5-4 DAYS, AND HENCE THE FAST WAS TERMINATED AT 3 DAYS. BY CONTRAST, IN SUMMER (JULY), ANIMALS REMAINED ALIVE UNTIL THE 8TH DAY, AND IN THIS GROUP

10.220.32.247 on June 15, 2017 http://ajplegacy.physiology.org/ Downloaded from
TABLE 1. Body weights and circulating substrates in fasting mice

<table>
<thead>
<tr>
<th></th>
<th>Days</th>
<th>0</th>
<th>1/3</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>7</th>
<th>8</th>
<th>16</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Body wt, g</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lean-winter</td>
<td>22.2</td>
<td>19.0</td>
<td>17.2</td>
<td>15.8</td>
<td>22.5</td>
<td>18.9</td>
<td>17.5</td>
<td>16.5</td>
<td>15.0</td>
<td>13.6</td>
</tr>
<tr>
<td>Lean-summer</td>
<td>±0.4</td>
<td>±0.4</td>
<td>±0.4</td>
<td>±0.3</td>
<td>±0.2</td>
<td>±0.2</td>
<td>±0.2</td>
<td>±0.2</td>
<td>±0.2</td>
<td></td>
</tr>
<tr>
<td>(17)</td>
<td>(17)</td>
<td>(17)</td>
<td></td>
<td></td>
<td>(10)</td>
<td>(10)</td>
<td>(10)</td>
<td>(10)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lean-summer</td>
<td>22.5</td>
<td>18.9</td>
<td>17.5</td>
<td>16.5</td>
<td>15.8</td>
<td>17.2</td>
<td>16.5</td>
<td>15.0</td>
<td>13.6</td>
<td>12.5</td>
</tr>
<tr>
<td>Lean-summer</td>
<td>±0.2</td>
<td>±0.2</td>
<td>±0.2</td>
<td>±0.2</td>
<td>±0.2</td>
<td>±0.2</td>
<td>±0.2</td>
<td>±0.2</td>
<td>±0.2</td>
<td>±0.2</td>
</tr>
<tr>
<td>(17)</td>
<td>(17)</td>
<td>(17)</td>
<td></td>
<td></td>
<td>(10)</td>
<td>(10)</td>
<td>(10)</td>
<td>(10)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lean-summer</td>
<td>22.5</td>
<td>18.9</td>
<td>17.5</td>
<td>16.5</td>
<td>15.8</td>
<td>17.2</td>
<td>16.5</td>
<td>15.0</td>
<td>13.6</td>
<td>12.5</td>
</tr>
<tr>
<td>Lean-summer</td>
<td>±0.2</td>
<td>±0.2</td>
<td>±0.2</td>
<td>±0.2</td>
<td>±0.2</td>
<td>±0.2</td>
<td>±0.2</td>
<td>±0.2</td>
<td>±0.2</td>
<td>±0.2</td>
</tr>
<tr>
<td>(17)</td>
<td>(17)</td>
<td>(17)</td>
<td></td>
<td></td>
<td>(10)</td>
<td>(10)</td>
<td>(10)</td>
<td>(10)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lean-summer</td>
<td>22.5</td>
<td>18.9</td>
<td>17.5</td>
<td>16.5</td>
<td>15.8</td>
<td>17.2</td>
<td>16.5</td>
<td>15.0</td>
<td>13.6</td>
<td>12.5</td>
</tr>
<tr>
<td>Lean-summer</td>
<td>±0.2</td>
<td>±0.2</td>
<td>±0.2</td>
<td>±0.2</td>
<td>±0.2</td>
<td>±0.2</td>
<td>±0.2</td>
<td>±0.2</td>
<td>±0.2</td>
<td>±0.2</td>
</tr>
<tr>
<td>(17)</td>
<td>(17)</td>
<td>(17)</td>
<td></td>
<td></td>
<td>(10)</td>
<td>(10)</td>
<td>(10)</td>
<td>(10)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lean-summer</td>
<td>22.5</td>
<td>18.9</td>
<td>17.5</td>
<td>16.5</td>
<td>15.8</td>
<td>17.2</td>
<td>16.5</td>
<td>15.0</td>
<td>13.6</td>
<td>12.5</td>
</tr>
<tr>
<td>Lean-summer</td>
<td>±0.2</td>
<td>±0.2</td>
<td>±0.2</td>
<td>±0.2</td>
<td>±0.2</td>
<td>±0.2</td>
<td>±0.2</td>
<td>±0.2</td>
<td>±0.2</td>
<td>±0.2</td>
</tr>
<tr>
<td>(17)</td>
<td>(17)</td>
<td>(17)</td>
<td></td>
<td></td>
<td>(10)</td>
<td>(10)</td>
<td>(10)</td>
<td>(10)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lean-summer</td>
<td>22.5</td>
<td>18.9</td>
<td>17.5</td>
<td>16.5</td>
<td>15.8</td>
<td>17.2</td>
<td>16.5</td>
<td>15.0</td>
<td>13.6</td>
<td>12.5</td>
</tr>
<tr>
<td>Lean-summer</td>
<td>±0.2</td>
<td>±0.2</td>
<td>±0.2</td>
<td>±0.2</td>
<td>±0.2</td>
<td>±0.2</td>
<td>±0.2</td>
<td>±0.2</td>
<td>±0.2</td>
<td>±0.2</td>
</tr>
<tr>
<td>(17)</td>
<td>(17)</td>
<td>(17)</td>
<td></td>
<td></td>
<td>(10)</td>
<td>(10)</td>
<td>(10)</td>
<td>(10)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lean-summer</td>
<td>22.5</td>
<td>18.9</td>
<td>17.5</td>
<td>16.5</td>
<td>15.8</td>
<td>17.2</td>
<td>16.5</td>
<td>15.0</td>
<td>13.6</td>
<td>12.5</td>
</tr>
<tr>
<td>Lean-summer</td>
<td>±0.2</td>
<td>±0.2</td>
<td>±0.2</td>
<td>±0.2</td>
<td>±0.2</td>
<td>±0.2</td>
<td>±0.2</td>
<td>±0.2</td>
<td>±0.2</td>
<td>±0.2</td>
</tr>
<tr>
<td>(17)</td>
<td>(17)</td>
<td>(17)</td>
<td></td>
<td></td>
<td>(10)</td>
<td>(10)</td>
<td>(10)</td>
<td>(10)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Values are means ± SE of the number of observations indicated in parentheses.

higher initially in the summer group, and the decline observed was less in magnitude (Fig. 1). Marked differences in plasma alanine and glutamine responses occurred (Fig. 2). The initial alanine values were higher in the summer group, though both decreased during the 1st day and showed a trend upward after the initial decline. However, in the winter group, a profound rise occurred on the 3rd day, just prior to the expected death of the animals, and such a change did not occur on day 7 in the summer group.

Again, for glutamine, the initial values were higher in summer, and, contrary to winter, a transient fall followed by a rise to initial values were observed. The winter group

7 days was taken as the limit for study. Obese mice, of the size employed, survived fasting more than 4 wk, but 16 days was chosen as the end point since the adaptation is considered to have reached a steady state by this time. At the latest time of sacrifice in both lean groups, there was no visible adipose tissue. In marked contrast, the obese mice at 16 days had abundant residual fat stores, still appeared clearly obese, and showed no signs that might be interpreted as due to vitamin deficiency.

A variation in the response of the lean mice between winter and summer was apparent in metabolic parameters, corresponding to the difference in survival time. Body weights were identical in the fed state, as was the loss over the first 2 days of fasting (Table 1). Thereafter, the summer group lost at a slower rate. In respect to circulating substrates, it is notable that the decline in glycemia was delayed, but to a comparable nadir in the summer group (Fig. 1). Among the important substrates for gluconeogenesis, differences in both absolute levels and in pattern of change were observed. Lactate and pyruvate levels were
showed an initial increase, followed by a second increase (at 3 days), analogous to that of alanine. Plasma glutamate, again lower in the winter at zero time, again showed an acute rise at day 3. No clear trend was apparent in the summer group.

Liver glycogen was determined only in the winter group. A progressive and uninterrupted decline occurred from 5.4 ± 0.7 g/100 g wet wt (mean ± SE, n = 7) at zero time to 2.0 ± 0.2 at 8 h, 0.72 ± 0.19 at 16 h, 0.41 ± 0.06 at 24 h, and remained at 0.09 ± 0.02 at 2 days and 0.06 ± 0.02 g/100 g wet wt at 3 days.

Metabolites, whose concentrations are considered to reflect the rate of fat mobilization, likewise showed important differences in the two lean groups (Fig. 3). An early doubling of FFA from already high levels, followed by a decline, was observed in summer. (Data for winter were not obtained.) Blood glycerol, higher in summer, showed a modest rise only at 4 days, whereas in winter, no change occurred, apart from a striking terminal fall. Both “ketone acids,” β-hydroxybutyrate and acetoacetate, were lower in the fed state, but rose to much higher fasting levels, which were sustained in summer. Again, a marked terminal decline occurred in the winter group. The total fat content of fed animals (summer) represented 18.9 ± 20% of body weight, or 3.45 g per mouse. By 3 days this had declined to 0.2 ± 0.3% or 1.2 g (n = 10 in each group). Such data were not obtained in winter.

The changes in circulating metabolites were associated with a high and unchanging rate of urine urea excretion in the winter group (Fig. 4). By contrast, there was a lower initial urea excretion, followed by a progressive decline in the summer. The cumulative total of urea so excreted over 3 days in winter was 144 mg, whereas it was 154 mg over 7 days in the summer. The excretion of urinary creatinine diminished progressively to about half of initial values in both groups, though it started at higher levels in winter.

Circulating hormone levels are shown in Table 2. A de

![Graph](http://ajplegacy.physiology.org/DownloadedFrom)
cline in IRI from comparable fed values occurred in both seasons. An initial rapid fall in both was followed by a plateau and an additional terminal decline. IRG values in winter showed no change, except the suggestion of a terminal rise, whereas in summer a slight, progressive decline occurred. Such alterations induced a downward shift in insulin/glucagon molar ratio with the onset of fasting, which reached a plateau in summer, but dropped sharply on day 3 in winter.

Turning now to the obob mice, differences in virtually all parameters were observed when compared to lean mice studied during the same season. Though of the same age, the initial body weight was almost double (Table 1). A loss of 3 g the 1st day was followed by a lesser rate of loss which stabilized at 0.5 g daily after 3 days. After 16 days, they were still heavier than the lean mice at the outset. Zero time plasma glucose was comparable (Fig. 1), but the decline was considerably slower in the obese, and reached hypoglycemic levels by 16 days. In relation to levels and trends of gluconeogenic precursors, a variety of differences was observed. Lactate and pyruvate values were comparably elevated initially and showed a trend downward with time (with the exception of a transient fall at 16 h). Whereas plasma alanine began considerably higher in the obese (Fig. 2) it dropped rapidly and tended to continue to decline thereafter. Completely different, the fed plasma glutamine was lower, and it rose progressively to 8 days, then declined slightly. Again, in contrast to the lean, the plasma glutamate was higher, declined until 8 days, then rose at 16 days.

The circulating fat-derived substrates all increased in concentration from zero time and remained elevated for the time intervals studied (Fig. 3). Whereas FFA began lower, its values never exceeded those seen in lean mice. This was not the case for glycerol and ketone acids, however (particularly β-hydroxybutyrate), which rose to concentrations that of the summer lean mice, but increased for 2 days, then the urinary urea excretion (Fig. 4), was initially identical to significantly higher and generally remained higher.

Turning now to the obob mice, differences in virtually all parameters were observed when compared to lean mice studied during the same season. Though of the same age, the initial body weight was almost double (Table 1). A loss of 3 g the 1st day was followed by a lesser rate of loss which stabilized at 0.5 g daily after 3 days. After 16 days, they were still heavier than the lean mice at the outset. Zero time plasma glucose was comparable (Fig. 1), but the decline was considerably slower in the obese, and reached hypoglycemic levels by 16 days. In relation to levels and trends of gluconeogenic precursors, a variety of differences was observed. Lactate and pyruvate values were comparably elevated initially and showed a trend downward with time (with the exception of a transient fall at 16 h). Whereas plasma alanine began considerably higher in the obese (Fig. 2) it dropped rapidly and tended to continue to decline thereafter. Completely different, the fed plasma glutamine was lower, and it rose progressively to 8 days, then declined slightly. Again, in contrast to the lean, the plasma glutamate was higher, declined until 8 days, then rose at 16 days.

The circulating fat-derived substrates all increased in concentration from zero time and remained elevated for the time intervals studied (Fig. 3). Whereas FFA began lower, its values never exceeded those seen in lean mice. This was not the case for glycerol and ketone acids, however (particularly β-hydroxybutyrate), which rose to concentrations that of the summer lean mice, but increased for 2 days, then the urinary urea excretion (Fig. 4), was initially identical to significantly higher and generally remained higher.

Turning now to the obob mice, differences in virtually all parameters were observed when compared to lean mice studied during the same season. Though of the same age, the initial body weight was almost double (Table 1). A loss of 3 g the 1st day was followed by a lesser rate of loss which stabilized at 0.5 g daily after 3 days. After 16 days, they were still heavier than the lean mice at the outset. Zero time plasma glucose was comparable (Fig. 1), but the decline was considerably slower in the obese, and reached hypoglycemic levels by 16 days. In relation to levels and trends of gluconeogenic precursors, a variety of differences was observed. Lactate and pyruvate values were comparably elevated initially and showed a trend downward with time (with the exception of a transient fall at 16 h). Whereas plasma alanine began considerably higher in the obese (Fig. 2) it dropped rapidly and tended to continue to decline thereafter. Completely different, the fed plasma glutamine was lower, and it rose progressively to 8 days, then declined slightly. Again, in contrast to the lean, the plasma glutamate was higher, declined until 8 days, then rose at 16 days.

The circulating fat-derived substrates all increased in concentration from zero time and remained elevated for the time intervals studied (Fig. 3). Whereas FFA began lower, its values never exceeded those seen in lean mice. This was not the case for glycerol and ketone acids, however (particularly β-hydroxybutyrate), which rose to concentrations that of the summer lean mice, but increased for 2 days, then the urinary urea excretion (Fig. 4), was initially identical to significantly higher and generally remained higher.

Turning now to the obob mice, differences in virtually all parameters were observed when compared to lean mice studied during the same season. Though of the same age, the initial body weight was almost double (Table 1). A loss of 3 g the 1st day was followed by a lesser rate of loss which stabilized at 0.5 g daily after 3 days. After 16 days, they were still heavier than the lean mice at the outset. Zero time plasma glucose was comparable (Fig. 1), but the decline was considerably slower in the obese, and reached hypoglycemic levels by 16 days. In relation to levels and trends of gluconeogenic precursors, a variety of differences was observed. Lactate and pyruvate values were comparably elevated initially and showed a trend downward with time (with the exception of a transient fall at 16 h). Whereas plasma alanine began considerably higher in the obese (Fig. 2) it dropped rapidly and tended to continue to decline thereafter. Completely different, the fed plasma glutamine was lower, and it rose progressively to 8 days, then declined slightly. Again, in contrast to the lean, the plasma glutamate was higher, declined until 8 days, then rose at 16 days.

The circulating fat-derived substrates all increased in concentration from zero time and remained elevated for the time intervals studied (Fig. 3). Whereas FFA began lower, its values never exceeded those seen in lean mice. This was not the case for glycerol and ketone acids, however (particularly β-hydroxybutyrate), which rose to concentrations that of the summer lean mice, but increased for 2 days, then the urinary urea excretion (Fig. 4), was initially identical to significantly higher and generally remained higher.

Turning now to the obob mice, differences in virtually all parameters were observed when compared to lean mice studied during the same season. Though of the same age, the initial body weight was almost double (Table 1). A loss of 3 g the 1st day was followed by a lesser rate of loss which stabilized at 0.5 g daily after 3 days. After 16 days, they were still heavier than the lean mice at the outset. Zero time plasma glucose was comparable (Fig. 1), but the decline was considerably slower in the obese, and reached hypoglycemic levels by 16 days. In relation to levels and trends of gluconeogenic precursors, a variety of differences was observed. Lactate and pyruvate values were comparably elevated initially and showed a trend downward with time (with the exception of a transient fall at 16 h). Whereas plasma alanine began considerably higher in the obese (Fig. 2) it dropped rapidly and tended to continue to decline thereafter. Completely different, the fed plasma glutamine was lower, and it rose progressively to 8 days, then declined slightly. Again, in contrast to the lean, the plasma glutamate was higher, declined until 8 days, then rose at 16 days.

The circulating fat-derived substrates all increased in concentration from zero time and remained elevated for the time intervals studied (Fig. 3). Whereas FFA began lower, its values never exceeded those seen in lean mice. This was not the case for glycerol and ketone acids, however (particularly β-hydroxybutyrate), which rose to concentrations that of the summer lean mice, but increased for 2 days, then the urinary urea excretion (Fig. 4), was initially identical to significantly higher and generally remained higher.
died with no visible adipose tissue, suggests that available triglyceride stores had been consumed. Thus at no point did these animals show the ability to convert quantitatively to the consumption of fat-derived substrates, in light of the unaltered high level of urea excretion. Indeed, the sharp elevation of plasma alanine and glutamine at 3 days could be viewed as an attempt of the organism to increase protein mobilization for conversion to glucose as an energy source to replace the declining availability of fat-derived fuels. Such a rise in amino acid concentrations could as well represent decreased uptake by the organs competent for gluconeogenesis. Nonetheless, the hypothesis of increased mobilization is favored by the stable blood glucose levels, since declining glucose synthesis might be expected to be accompanied by hypoglycemia. Adibi (1) has shown a similar early fall in circulating alanine and glutamine in 8-day-fasted rats, though no terminal rise in concentration was observed. Death could well have supervened in the mice as the result of excessive protein loss, which has been shown to be associated with death from malnutrition in man (18). The early fat, amino acid, and probably glycogen mobilization is likely to have been primarily regulated by falling insulin levels and by the interaction of insulin and glucagon levels (29). The later response, associated with decline of fat-derived substrates and rise in amino acids, remains unexplained on a hormonal basis. It may be related to a direct effect of free fatty acids and/or ketones on muscle amino acid metabolism (7, 8).

These terminal responses of the lean mouse are of interest in light of the usual link between hepatic ketogenesis and gluconeogenesis in low-insulin states (16). If the rise in amino acids represents an outpouring from muscle in an attempt to maintain the flow of substrate (as glucose) for oxidation, a concurrent increase in hepatic gluconeogenesis might be predicted, but in a situation in which the energy supply for this process can no longer come from fatty acid oxidation. An analogous adaptation has been demonstrated in the fasted newborn rat, in which gluconeogenesis is inadequate to maintain glycemia, due probably to the absence of fatty acids despite adequate glucogenic substrate and activity of appropriate enzymes (19).

The seasonal alteration in response in lean mice consisted of the appearance in the summer of the ability to mobilize fat to a greater extent, resulting in ketosis of greater magnitude and longer duration, coupled with the ability to decrease protein mobilization, and hence prolongation of survival. Such a finding is explained neither by differences in levels and relationships of the hormones assayed nor by environmental or dietary changes, since stringent efforts were made to keep these constant. This does not, however, preclude some unrecognized technical artifact, though it is considered unlikely. Studies of body composition changes with season are unavailable for this strain. However, it has long been appreciated that rat adipose tissue responds to stimuli of lipolysis in vitro to a markedly greater extent in summer than in winter (24), though the mechanism is unknown. Hence it is possible that an analogous in vivo effect is operative in the present study. Interestingly, the total amount of urea excreted in the two seasons was equivalent, though spread over twice the duration of time in summer. This suggests that the mobilization of the same critical amount of protein in both seasons is an important limiting factor in survival and that this amount is not a function of the season.

It is noteworthy that the pattern of plasma amino acid response in this situation in which some protein sparing occurs is not equivalent to that observed in man with respect to alanine (13, 14) or glutamine (7). In man, alanine falls precipitously, then continues to drift downward, whereas glutamine shows a progressive decline with time. Such changes have been shown to be due to curtailment of muscle release (12, 28). Hence, the differing pattern of response in the summer group of mice could reflect a different mechanism of protein conservation. Since neither amino acid turnover nor metabolism of individual tissues has yet been studied, however, inferences regarding mechanism are unwarranted. Though recent studies have demonstrated that blood cells participate in interorgan transport of amino acids (2, 3, 15), the patterns of response in the present study in which whole blood alanine was measured as well (unpublished data) were identical to those presented for plasma.

Whereas the lean mouse in summer was able to achieve a degree of prolongation of survival, compared with its response in winter, the obob mutant was able to achieve a highly successful adaptation consistent with prolonged survival. This was accomplished by a pattern of protein mobilization identical to that observed in man, namely, early mobilization followed by marked curtailment. It is of interest that the magnitude of urea excretion was at no time as great as that of the lean mice in winter, and the cumulative total over a period 5 times longer was less than twice. (The magnitude of such mobilization in the mouse must be considered an underestimate, since total urine nitrogen was not determined, and hence, the nephrogenic component, ammonia nitrogen, which increases in man to maintain acid-base homeostasis, is not included.)

It was of interest that the pattern of creatinine excretion was different in the obese mice from the lean. In the lean mice, as has been shown in the rat (unpublished data), it decreased steadily throughout the fast. In the obese, however, it remained largely unchanged up to 16 days of fasting (Fig. 1). If it is true in the mouse that creatinine excretion is an index of muscle mass and activity, this finding suggests that the lean mice has lost more muscle mass during the fast than the obese, suggesting that the obese mice may derive a portion of the gluconeogenic amino acids from tissues other than muscle.

Of great importance in this adaptation is the rise in FFA and the higher and more sustained levels of blood ketone acids in the obese. The inference is that, as has been shown for man (31), the obob mouse is able to conserve protein by the conversion of the central nervous system to ketone-body oxidation from that of glucose. The appropriate alterations in brain enzymes have been shown in the fasted rat (35). Furthermore, ketone bodies have recently been demonstrated to be major metabolic fuels for the brain in suckling and adult rats (20) and the rate of their oxidation has been demonstrated to be a function primarily of their circulating concentrations in a number of species, including the mouse (40).

The demonstration that the obob mouse is capable of mobilizing triglyceride stores is not considered to exclude a
relative defect in this process contributing to the etiology of the syndrome of obesity. It does indicate that there is no absolute defect in fat mobilization, since the latter would preclude survival of the fasted state. It is to be emphasized that the responses observed must be considered typical for the ob ob gene present on the C57BL/6 background. Extrapolations to animals with the same genetic alteration present on different or heterogeneous background strains are not warranted. Preliminary studies (unpublished data) of other varieties of obesity in experimental rodents have, however, shown that representatives of the dbM mutant, of Acomys (the spiny mouse), and obesity due to goldthioglucose treatment are all capable of surviving prolonged periods of starvation.

Again, the response of plasma amino acids differs both from that observed in man (particularly for glutamine) and the lean rat. Since all patterns were consistent with protein conservation, it is apparent that further studies are required to elucidate the various possible mechanisms.

It is of note that the plasma IRG of the obese did not differ considerably from that of the lean, either fed or with fasting. This contributes to an exceedingly high insulin/glucagon molar ratio, thus far demonstrated only in the fetal rat (19) and considered to be one of maximal anabolism. Even with the fall seen after 2 days of fasting, the ratio remains greater than that in the fed lean mice. It is conceivable that such a ratio may contribute importantly to the pathogenesis of the syndrome of obesity. Abnormalities of glucagon secretion have recently been reported in obese man (23, 41), though the results have been conflicting, and the mechanisms for either result have not been clearly defined.

The pancreatic content of IRI observed in both lean and obese fed mice is consistent with previous observations (37), though data for animals of the specific age employed are not available. The changes during fasting are of considerable interest, being a reflection of the rates of synthesis, secretion, and perhaps intrapancratic degradation. Though such static data do not allow for interpretation of the rates of these phenomena, it is of interest that at 8 wk, in the presence of exceedingly high plasma IRI, the pancreatic content is lower than for lean animals. This reflects a very rapid rate of biosynthesis and secretion. However, when secretion is acutely suppressed in fasting, a marked increase in content occurs, which is sustained for a long period of time. By contrast, in the lean, as in the rat (27), little acute change in content is followed by the expected decline, probably reflecting a rapid diminution of protein synthesis, as found in the pancreas of fasted rats (30), this being associated with a decreased secretion of insulin (6). The findings in the obese suggest that, whatever the cause of hypersecretion, the feedback in relation to biosynthesis is less sensitive to situations of decreased secretion. The changes in pancreatic IRG content over 7 days were also opposite in direction in the lean and obese. Toward the end of the fasting period in both, however, the values were lower than in fed mice. The acutrise in the obese might again be associated with the decrease in plasma levels observed at 2 days.

REFERENCES


Present address of D. P. Cameron: Medical Research Centre, Prince Henry's Hospital, Melbourne, Australia.

Present address of E. B. Marlls: Room 5242, Medical Sciences Building, University of Toronto, Toronto, Ontario, Canada.
RESPONSES OF LEAN AND OBESE MICE TO FASTING


