Simultaneous Determination of Regression Equations for Body Composition, Body Measurements and Metabolic Rate in Rats. (32591)

ROBERT J. T. JOY,* ROBERT F. KNAUFT, JEAN MAYER
Department of Nutrition, Harvard University School of Public Health, Boston, Massachusetts

As part of a series of studies of the effect of chronic cold exposure on the growth of the rat, we found it necessary to secure simultaneous measurement of the variables of body composition, body size and measurement, and metabolic rate on a number of normal rats over an extended weight range. A recent extensive symposium(3) suggested that although many such data were available, in no instance were they all secured simultaneously from the same population and in few instances did they extend to the older (and heavier) rat. Further, our methods for determining total body lipid(12) allowed a more accurate estimate of non-lipid components than older methods. These "normal rat" values are thus reported here.

Methods. 56 male rats, of a modified Sprague-Dawley strain (Charles River) were measured in groups of 5 over a weight range of 180 to 650 grams. All rats were fed standard Purina rat diet (3.60 kcal/g), and were individually caged in a room at 25°C ambient temperature. Daily weight and food intake were measured to $\pm$ 0.5 g(7).

On the day of measurement, 5 animals were selected at random, weighed, fasted for 4 hours and reweighed. Oxygen consumption was then measured in a modified open circuit system(5) by pumping room air (200 cc/min) through a glass cylinder selected for size such that the rat occupied 60-70 percent of its volume. The air stream was then led through tubes of indicating calcium sulfate (Dririte), Ascarite, a paramagnetic oxygen analyzer (Beckman, Model E-2), and exhausted through a 3 liter wet test meter (Precision-Scientific). Three 10-minute measurements were taken and averaged. All volumes were reduced to STPD. Weighing of the pre-tared Ascarite tubes provided CO_2 production data for calculation of the respiratory quotient (R.Q.).

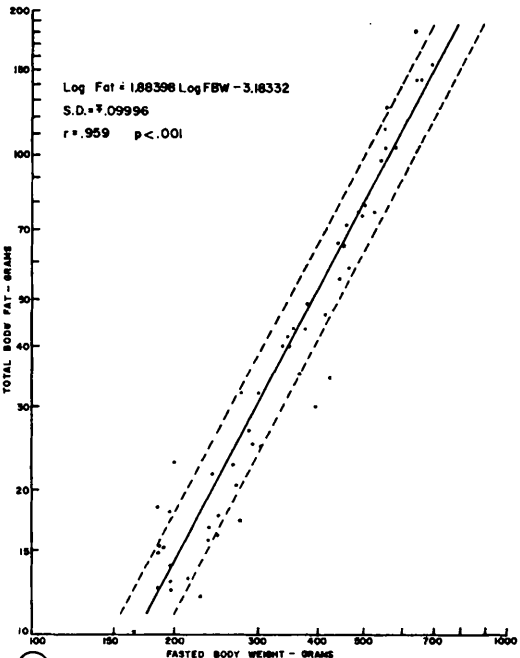
The animals were weighed again and anesthetized with sodium pentobarbital (4 mg/100 g, i.p.). When anesthesia was complete, measurements were taken of the nose-anus length, and the circumferences of the chest (midway on sternum), and abdomen, (midway between rib cage and iliac crest). The animal was then reweighed, the hair removed first with clippers and then calcium thioglycollate, the latter was washed off, the animal dried with a towel and weighed again. The last 2 weight differences were taken as the hair weight.

The rat was then immersed in liquid nitrogen, divided in segments and passed through a grinder into a 2:1 chloroform: methanol solution (10 ml/mg of rat). The tissue was extracted twice for 30 minutes each time, and filtered through a Buchner funnel with a sintered glass filter. After total volume determination an aliquot of the filtrate was separated into lipid and non-lipid fractions by partition chromatography on Sephadex G-25 columns as described by Therriault and Poe (12). The solid residue in the Buchner funnel was dried under vaccum, weighed, and a portion retained for analysis of nitrogen content by the semi-micro Kjedahl method, which was corrected to protein by multiplying by 6.25. Aliquots of the hair clippings were treated in the same way. All analyses were done in duplicate and averaged.

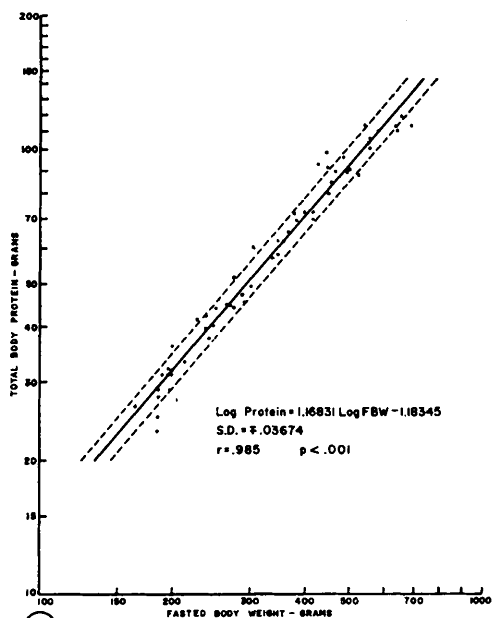
After appropriate weight and volume corrections, the lipid, non-lipid, and dried filtered residue weights were summed and substracted from the nude weight of the rat—this weight was taken as the body water content. The fat (lipid), protein, and non-lipid values reported are for the sum of the values found for nude carcass and the hair.

Data were regressed(4) against the 4-hour fasted body weight and 4 equations (linear, semi-logarithmic and log-log) tested for best fit by use of an IBM 1620 computor. The equations reported are those with the highest correlation coefficients.

* Present address: USA Research Institute of Environmental Medicine, Natick, Massachusetts.



(1)



(2)

FIG. 1. Allometric relationship between 4-hour fasted body weight and total body lipid for 56 rats. S.D. is 1 standard deviation of the estimate.

FIG. 2. Allometric relationship between 4-hour fasted body weight and total body protein for 56 rats. S.D. is 1 standard deviation of the estimate.

Results. Fig. 1, 2, and 3, present the relationships between the 4-hour fasted body weights and the total body fat (lipid), total body protein and the total hair weight. Table I summarizes the regression formulae for the other body composition and body size variables measured.

Fig. 4 presents the regression equation for oxygen consumption (as kcal/24 hr). The R.Q. was 0.937 (± S.S. 0.023) and a conversion factor of 4.973 was used to convert oxygen consumption to kilocalories.

Analysis of the hair showed mean values of 3.58 percent (of total weight) for lipid, 5.92 percent for non-lipid, and 84.74 percent for protein.

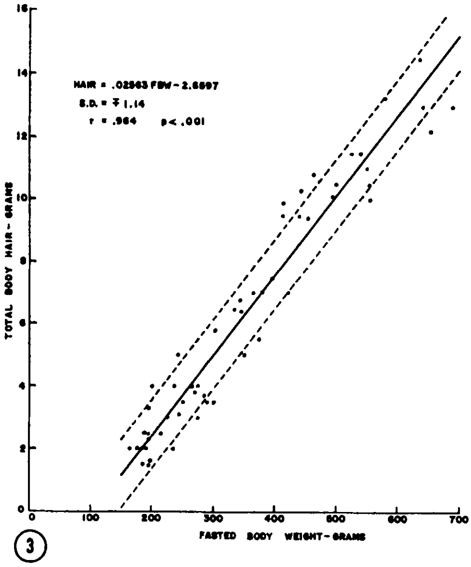
Discussion. Several reviews have discussed at length the appropriate concepts and mathematical presentations of body composition and its relationship to growth and body size(2,6,8, 10,14). Our purpose in this paper is to report normative values for a population sample of adequate size and of somewhat heavier body weights than usually presented.

The equations reported are those with the highest correlation coefficients. With the exception of nose-anus length, the rodentometric measurements gave slightly higher correlation

TABLE I. Formulae Coefficients (a and b), Standard Deviations of the Estimate (S.D.) and Coefficients of Correlation (r) for the Regression of the Listed Variables (Y) upon the 4-Hour Fasted Body Weights (X) of 56 Rats, for the Equations:

$$^{*}\log Y = \log a + b \log X$$
$$^{\dagger} Y = a + b X$$

Variable	a	b	± S.D.	r
Water (g)*	+0.31674	0.79791	0.03536	0.971
Non-lipid (g)*	−1.32269	0.76538	0.20288	0.726
Nose-anus length (cm)*	+0.53731	0.31553	0.01319	0.976
Chest circumference (cm)†	+7.42401	0.01533	0.9432	0.921
Abdomen circumference (cm)†	+9.83819	0.01794	0.8849	0.948



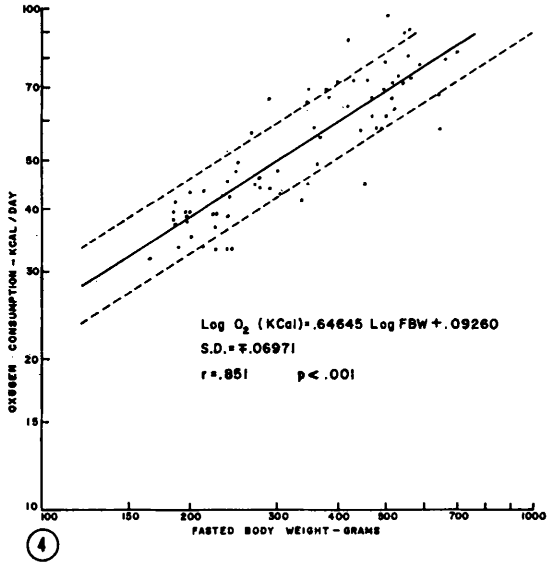


FIG. 3. Linear relationship between 4-hour fasted body weight and total body hair for 56 rats. S.D. is 1 standard deviation of the estimate.

FIG. 4. Allometric relationship between 4-hour fasted body weight and resting metabolic rate in 56 rats. The R.Q. was 0.937 and 4.973 was used as a conversion factor to kcal. S.D. is 1 standard deviation of the estimate.

coefficients with the linear estimating equation. However, the allometric equation (log-log) correlation coefficients were only slightly lower (chest: 0.908, and abdomen: 0.943), while the correlation coefficient for nose-anus length in the linear equation was 0.957. Similarly, the allometric equation for hair had a correlation coefficient of 0.953. We suggest that these small differences reflect the inherent errors of measurement of these variables rather than profound implications for the "laws of growth"(2,8).

The analysis of total body fat (lipid) by partition chromatography improves the estimate of mono-, di-, and triglycerides by 5-10 percent(12). The non-lipid components (salts, peptides, amino acids, carbohydrates, purines and pyrimadines) are included in the determination of total fat in the older Soxhlet methods(9,11)—an error that becomes even more significant if these values are used to estimate carcass calorie content for food efficiency studies(2,9).

Our data for hair composition—at least for protein—agree very closely with the literature values(1). It should be noted that even though total hair is a small percent of body weight (less than 2%), the equations for fat, protein, and non-lipid include the hair values, while the body water data are expressed in terms of hair-free four-hour fasted body weight.

There has been discussion in the literature about an appropriate variable for relating body composition to metabolic rate(2,8,10, 13). It is of interest to note that in regressing metabolic rate upon fat-free body weight, the best estimate was given with the allometric equation with a correlation coefficient of 0.830. When metabolic rate was similarly regressed upon total body protein, the correlation coefficient was 0.810. From the data presented here, it appears that the 4-hour fasted body weight provides as good or better an estimate of metabolic rate and is obviously easier to determine for a given experimental population.

Summary. 56 normal male albino rats, with body weights from 180 to 650 grams were analyzed for total body fat (lipid), protein, non-lipid, and water, and had measurements made of their oxygen consumption, nose-anus length, and chest and abdomen circumferences. All these variables were re-

gressed upon the 4-hour fasted body weight and the appropriate regression equations determined.

We are grateful to Mr. Raymond Neff for the computer analysis and to Miss Elizabeth Loutrell and Mr. James Richards for expert technical assistance. These studies were supported in part by a grant-in-aid from the National Institute of Arthritis and Metabolic Diseases (AM 02911), National Institutes of Health, US Public Health Service; and by the Fund for Research and Teaching, Department of Nutrition, Harvard School of Public Health.

1. Block, R. J., Bolling, D., The Amino Acid Composition of Proteins and Foods, 1951, C. C Thomas, Springfield, Ill.

2. Brody, Bioenergetics and Growth, 1945, Reinhold Corp., New York.

3. Brozek, J., Behnke, A. R., Ann. N.Y. Acad. Sci., 1963, v110, 1-1018.

4. Croxton, F. E., Elementary Statistics and Applications in Medicine and the Biological Sciences, 1959, Dover, New York.

5. Depocas, F., Hart, J. S., J. Appl. Physiol., 1957, v10, 388.

6. Huxley, J. S., Problems of Relative Growth, 1932, Methuen and Co., London.

7. Joy, R. J. T., Mayer, J., J. Appl. Physiol., 1967 (in press).

8. Kleiber, M., The Fire of Life, 1961, John Wiley and Sons, New York.

9. Mayer, J., Vitale, J. J., Am. J. Physiol., 1957, v189, 39.

10. Miller, I., Weil, W. B., Jr., Ann. N.Y. Acad. Sci., 1963, v110, 153.

11. Rathbun, E. N., Pace, N., J. Biol. Chem., 1945, v158, 667.

12. Therriault, D. G., Poe, R. H., Canad. J. Biochem., 1965, v43, 1427.

13. von Döbeln, W., Acta Physiol. Scandinav., 1956, v37, Suppl. 126.

14. Zucker, L., Zucker, T. F., J. Gen Physiol., 1941, v25, 445.

Received August 28, 1967. P.S.E.B.M., 1967, v126.

Further Characterization of Specific Heterophile Antibody from Infectious Mononucleosis Serum.* (32592)

KOSAKU YOSHIDA† AND ANDRÉ NAHMIAS‡
(Introduced by Morris Tager)
Department of Pediatrics, Emory University School of Medicine, Atlanta, Georgia

The biochemical and immunological characteristics of immunoglobulins belonging to the IgM class have been reported for certain disease conditions, such as multiple myeloma, chronic hemolytic anemia and Waldenstrom's macroglobulinemia(1-8). In a previous contribution to this journal(9) we reported on the isolation and purification of the specific heterophile antibody from infectious mononucleosis. In the present report, further observations on the characterization of the heterophile antibody with particular emphasis on differences between the IgM of the heterophile antibody and non-heterophile antibody IgM are presented.

† Present address: Dept. Microbiology, Northwestern Univ., Chicago, Ill.

* Supported by Grants AI-06157 and 3-T01-AI 00304 from Nat. Inst. Health, USPHS.

‡ André Nahmias is recipient of a Research Career Development Award from the USPHS.

Materials and methods. Isolation of heterophile antibody (HA). As previously described(9) HA was isolated by adsorption with beef cell antigen of serum from patients with infectious mononucleosis; this serum had been submitted to prior adsorption with guinea pig kidney antigen. The heterophile antibody was dissociated from the beef cell antigen by an ether elution method and was further purified by sucrose density gradient ultracentrifugation. The purified concentrated eluates, when tested by the double diffusion agar method and immunoelectrophoresis, were noted to give only 1 line with goat antihuman IgM serum.

Electrophoresis of purified HA. The purified eluate was tested by cellulose acetate strip electrophoresis using LKB apparatus. Cellulose acetate strips (S & S, Serometrics, Inc.) of 18 × 2.4 cm size were used with the Tris (80.0 g/l), boric acid (8.0 g/l), EDTA (6.0