Energy Intake and Utilization Vary During Development in Rats[1]

(Manuscript received 7 August 1998. Initial review completed 23 November 1998. Revision accepted 10 May 1999.)

Susanna Iossa, Lillà Lionetti, Maria Pina Mollica, Antonio Barletta and Giovanna Liverini[2]

Department of General and Environmental Physiology, University of Naples "FEDERICO II", Italy I-80134

ABSTRACT Energy intake, utilization, and partitioning were determined in male Wistar rats from 25 to 180 d of age. Serum free triiodothyronine, leptin, and free fatty acid concentrations were also measured. Energy balance measurements allowed us to identify a period from 25 to 90 d, characterized by a rapid body growth rate and another from 90 to 180 d, during which body growth rate slowed. From 25 to 180 d, we found decreases in daily energy intake and expenditure, which were faster before 90 d. The first period was characterized by storage of lipid and protein. In the second period, protein deposition approached zero and the excess of ingested energy was entirely stored as fat, so that age-associated obesity began to develop. The inability of rats to maintain a stable body weight after the cessation of growth of lean body mass is not due to decreased resting metabolism but rather to a partial leptin resistance. J. Nutr. 129: 1593–1596, 1999.

KEY WORDS: • energy balance measurements • resting metabolic rate • leptin • developing rats

Coordination of energy intake and energy expenditure is involved in the regulation of body weight: obesity, which is defined as a body fat excess, can be caused by long-term alterations in energy balance. In general, when food intake exceeds energy expenditure, the retained energy is deposited as fat. To maintain energy balance, the body is capable of changing its metabolic rate. In young rats when energy intake exceeds requirement, metabolic rate may increase and some of the excess energy is released in the form of heat (Iossa et al. 1995, 1997, Lionetti et al. 1996a, 1996b, Liverini et al. 1994, 1996). On the other hand, both humans and rodents develop a spontaneous obesity as they grow older (Barzilai and Rossetti 1995, Masoro 1980, Newby et al. 1990). Thus, in the older rats, some regulatory mechanism which allows maintainance of body weight can fail.

In an attempt to gain further insights into the energetic aspects of the tendency to accumulate fat typical of rats as they grow old, we evaluated the relative importance of changes in energy intake and expenditure in developing rats. Since, to our knowledge, a complete study on the energy utilization in maturing Wistar rats has not been done, we carried out measurements of energy balance and changes in body energy compartments in young rats in which body fat is quite low. These animals were then followed until adult age when the obesity propensity starts to manifest. In addition, to have more information about the role of energy metabolism in body weight control, resting metabolic rate (RMR)[3] was measured after 16 h of food deprivation by using indirect calorimetry. Variations in serum free triiodothyronine (FT_3), leptin, and free fatty acids (FFA) were also checked.

METHODS

The animals studied were male Wistar rats obtained from Charles River (Calco, Como, Italy) just after weaning. They were individually housed in metabolic grid-bottomed cages at 24°C under an artificial circadian 12-h light/12-h dark cycle with free access to a standard stock diet (Mucedola 4RF21; Settimo Milanese, Milan, Italy) and water. The composition (% energy) of this diet was protein 29.0, lipid 10.6, and carbohydrate 60.4; its gross energy density was 15.88 kJ/g wet food. Animal care, housing, and killing met the guidelines of the Italian Health Ministry.

At the start of the study, 28 rats (aged 25 d) were divided into seven groups, each composed of four rats, with similar mean body weight. One group was killed for the estimation of energy content and body composition of 25-d-old rats. The other six groups were killed at 40, 55, 90, 120, 150, and 180 d of age. At each time of death, the killed animals had a mean and range of body weight equal to that of the other groups of animals. Therefore, in our calculations of energy gain, we assumed that the surviving animals contained the same proportions of fat, protein, and water in their carcasses as those in the killed animals.

The day before the killing, the rats of each group were food-deprived for 16 h (from about 1700 h), and the following morning they were used for RMR determination. The oxygen consumption was measured in an open-circuit oxygen consumption system (O_2-ECO; Columbus Instruments, Columbus, OH), designed to monitor oxygen consumption in small animals. The instrument was calibrated with room air before and after each measurement. Although most rats quieted down after about 30 min in the chamber, all rats were allowed to adapt to the experimental environment for a minimum of 60 min before beginning the measurements. RMR in food-deprived rats was measured in a chamber at 24°C over a period of at least 10 min during which the rat remained quiet.

Measurement of body composition. After RMR measurements, the rats were anesthesized with chloral hydrate (40 mg/100 g body wt), and blood was collected via the inferior caval vein. Then, the carcasses were weighed, autoclaved for 90 min, chopped into small pieces, thoroughly mixed, and finally homogenized with water (volumes equal to twice the carcass weight) in a Polytron homogenizer. Aliquots of the homogenate were analyzed for lipid and water content. Lipid content was determined gravimetrically after extraction in chloroform–methanol and evaporation to constant

[1] This work was supported by Ministero dell'Università e della Ricerca Scientifica e Tecnologica of Italy.

[2] To whom correspondence should be addressed at Dipartimento di Fisiologia Generale ed Ambientale, Via Mezzocannone 8, I-80134, Napoli, Italy. Fax: +39-081-5426868. E-mail: susiossa@unina.it

[3] Abbreviations used: ANOVA, analysis of variance; FFA, free fatty acid; FT_3, free triiodothyronine; ME, metabolizable energy; RMR, resting metabolic rate.

weight by a rotating evaporator (Heidolh, Germany) by the method of Folch et al. (1957). The energy as lipid was calculated from the lipid content by using the coefficient of 39.2 kJ/g for the energy content of lipid. Water content was determined by the difference in weight of the homogenate before and after drying at 70°C in a vacuum oven. Then, small pellets (about 200 mg) of the dried homogenate were made, and the body energy content was measured with a bomb calorimeter (Parr adiabatic calorimeter; Parr Instruments Co., Moline, IL). The energy as protein was calculated from a general formula relating lipid-derived energy, total energy value of the carcass, and protein-derived energy. The formula is based on the fact that lipid and protein are the only energy-yielding components of the carcass with a negligible (<1%) contribution from carbohydrates (Dulloo et al. 1990). Protein content was obtained by using the value of 22.4 kJ/g for the energy content of protein.

Energy balance measurements. Body weights and food intakes were monitored daily to allow calculations of body weight gain and gross energy intake. The feces and spilled food were also collected daily, dried, and ground to a powder before determining their energy content with the bomb calorimeter. The gross energy content of the stock diet was also determined by bomb calorimetry.

Digestible energy intake was obtained by subtracting the energy measured in the feces and spilled food from the gross energy intake as measured from daily food consumption. Metabolizable energy (ME) intake was expressed as digestible energy intake × 0.96 (Barr and McCracken 1984). The gain in energy was obtained from the difference between the final body energy content at the end of each period and the energy content of the animals killed at the end of the previous period. Energy expenditure was calculated from the difference between ME intake and energy gain.

Serum metabolites. Serum samples were stored at −20°C until the time of measurement. Serum FT_3 and leptin levels were measured using commercial radioimmunoassay kits (Coat A Count; Diagnostic Products Corporation, Los Angeles, CA for FT_3, and Mediagnost, Germany for leptin). Inter- and intraassay coefficients of variation were 7 and 4% for T_3 assay and 6 and 9% for leptin assay. Serum FFA levels were measured by colorimetric enzymatic method using a commercial kit (Boehringer Mannheim, Italy).

Statistical analysis. Data are given as means ± SEM of four different rats. Statistical analyses were performed by one-way analysis of variance (ANOVA). Post-hoc comparison between group pairs was made with the Tukey test after ANOVA had established significant differences among groups. Regression analyses were also performed. Probability values less than 0.05 were considered to indicate a significant difference. All analyses were performed using GraphPad Prism (GraphPad Software, San Diego, CA).

RESULTS

Body composition. Body weight and body energy/g body wt of developing rats from 25 to 180 d of age are reported in **Figure 1**A. Before the sexual maturity, an initial rapid growth period occurred; then, the gain in body mass slowed. Body energy/g body wt significantly increased between 40 and 55 d of age; a further increase occurred between 120 and 150 d of age. Body water decreased with age, from about 73% of body weight in the youngest rats to about 60% in the oldest rats (Fig. 1B). The percentage of the body protein did not significantly vary with age, although there was a tendency ($P = 0.07$) to decrease in rats of 150 and 180 d of age. Percent body lipid significantly increased in developing rats, from about 5% in 25-d-old rats to about 17% in 180-d-old rats (Fig. 1B).

Energy balance and metabolic rate. Both daily ME intake and energy expenditure expressed per metabolic body size ($kg^{0.75}$) significantly decreased in developing rats up to 90 d of age and remained constant thereafter (**Fig. 2**A). RMR expressed per g body protein exhibited the highest value in 25-d-old rats. Then, RMR significantly

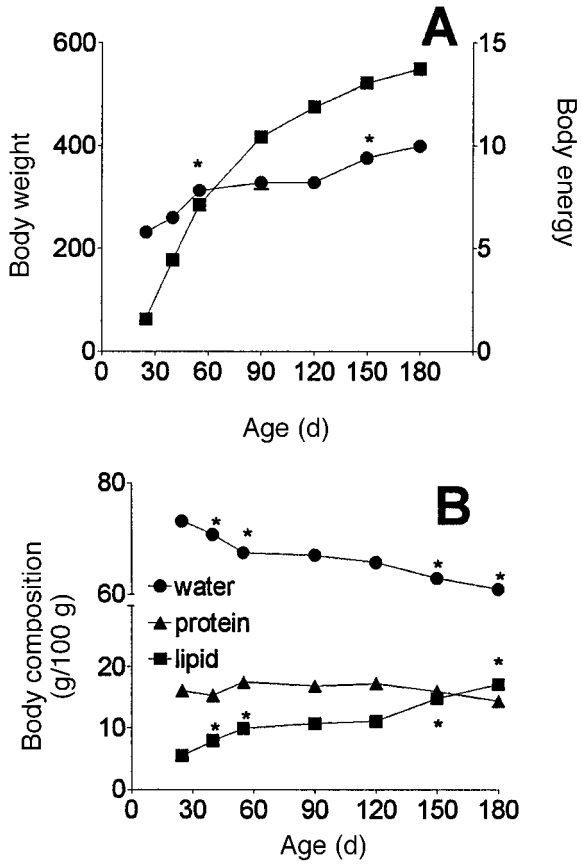

FIGURE 1 (*A*) Body weight and energy; ■, g; kJ/g, ● and (*B*) Body composition at different ages in Wistar rats. Each point represents mean ± SEM, $n = 4$. *$P < 0.05$ compared to the preceding age (not reported for body weight values).

decreased, reaching a constant value from 55 to 180 d of age (Fig. 2A).

Partitioning of ME intake in rats at different ages is reported in Figure 2B. The total cost of storage was determined taking into account that the energy loss in storing 1 kJ of protein is 1.25 kJ (Pullar and Webster 1977), while the corresponding energy cost for fat deposition is 0.36 kJ/kJ for diets with a high percentage of carbohydrates (Pullar and Webster 1977), such as the diet used here. The percentage of ME intake used for storage of protein and lipid significantly decreased with age. Expressed as a percentage of ME intake, values obtained for energy expenditure excluding the total cost of storage, corrected energy expenditure, significantly increased in developing rats up to 120 d of age and remained unchanged thereafter. The percentage of energy intake stored as protein significantly decreased with age, while the percentage stored as fat reached the lowest value at 120 d of age (Fig. 2B).

The percentage of ingested protein which was stored as carcass energy significantly decreased, especially after 90 d of age, while the percentage of ingested lipid which was stored reached the lowest value at 120 d of age and then increased in 150 and 180-d-old rats (Fig. 2C).

Serum metabolites. Serum FFA significantly increased only in 180-d-old rats (**Fig. 3**A). Serum FT_3 showed a peak at 55 d of age and did not significantly vary thereafter. Serum leptin levels significantly increased in developing rats, reaching a constant value at 150 and 180 d of age. Leptin levels were strongly correlated with body fat mass

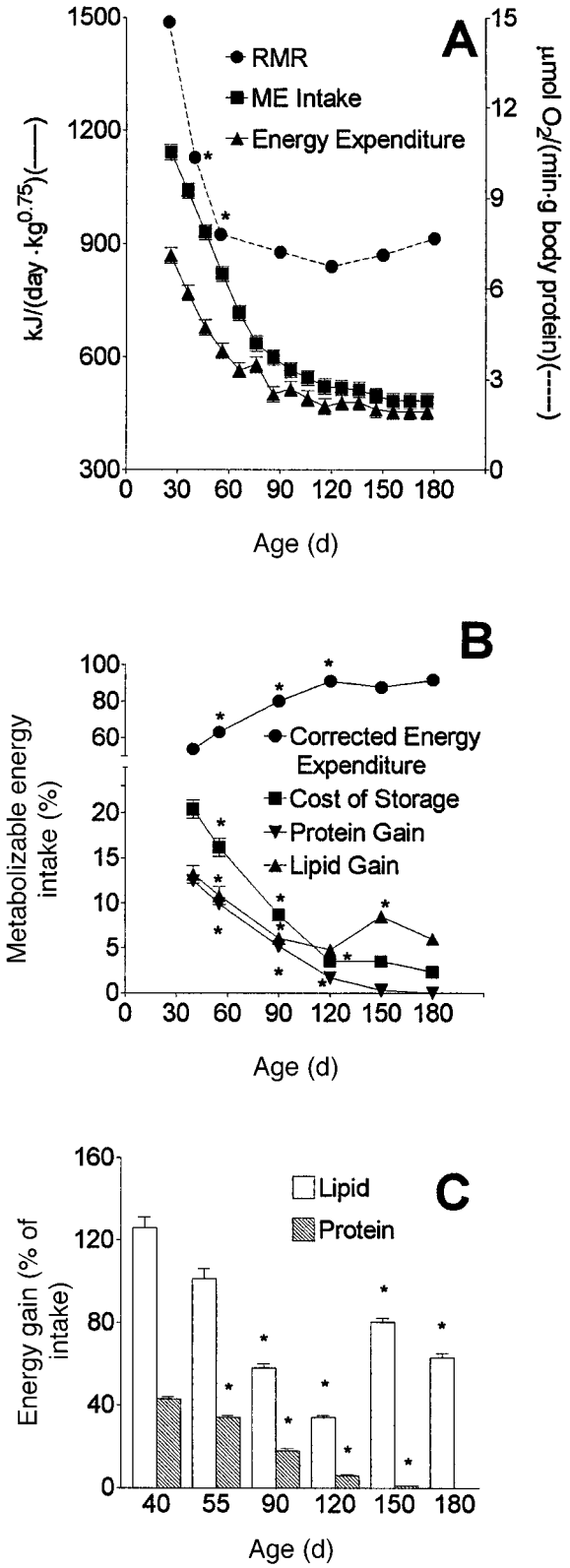

FIGURE 2 (*A*) Daily metabolizable energy (ME) intake, daily energy expenditure, and resting metabolic rate (RMR). (*B*) Partitioning of ME intake, (*C*) percent of ingested protein, and lipid stored in the body at different ages in Wistar rats. RMR was measured at 24°C in rats food-deprived for 16 h. Each point represents mean ± SEM, $n = 4$. *$P < 0.05$ compared to the preceding age.

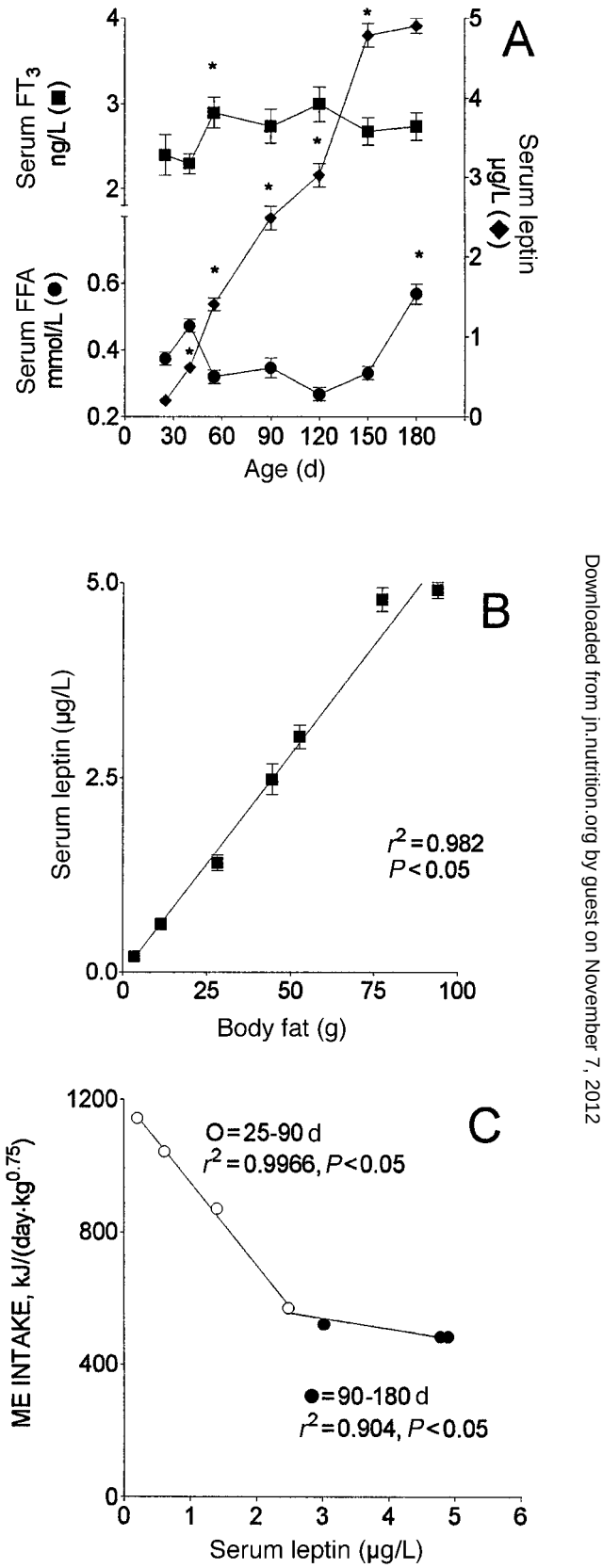

FIGURE 3 (*A*) Serum concentration of free triiodothyronine (FT_3), free fatty acids (FFA), and leptin. (*B*) Correlation between serum leptin and body fat. (*C*) Correlation between serum leptin and metabolizable energy (ME) intake in Wistar rats at different ages. Each point represents mean ± SEM, $n = 4$. *$P < 0.05$ compared to the preceding age.

(Fig. 3B) and daily ME intake/kg$^{0.75}$ (Fig. 3C). However, regression lines for the correlation between leptin levels and ME intake showed significantly different slopes during the period 25–90 and 90–180 d.

DISCUSSION

Rat maturation is characterized by variations in energy partitioning. A rapid body growth rate (Fig. 1), coupled with a significant storage of body protein (Fig. 2), was followed by a lower rate of growth, with protein deposition that approached zero (Fig. 2). However, ME intake remained about 10% higher than the energy needs for maintenance (Fig. 2). This excess of energy was entirely stored as fat (Fig. 2). Therefore, the ingested proteins, which in rapidly growing rats were partly stored in the body and partly used to replace stores (Fig. 2), were at this time used only to support protein turnover. In agreement, we can calculate that protein oxidation contribution to total energy expenditure goes from about 20% in 40-d-old rats to about 30% in 180-d-old rats.

Up to 55 d of age, body energy gain as lipid and lipid intake was almost equivalent, while starting from 55 d of age lipid gain represented only a part of lipid intake (Fig. 2). It follows that an increase in fat utilization for metabolic needs takes place at this stage, although serum FFA levels increased only in 180-d-old rats (Fig. 3). Therefore, seemingly due to reduced energy intake from 55 d of age, carbohydrate and protein oxidation was not sufficient to meet body energy needs (Fig. 2).

The constant reduction in energy intake during development (Fig. 2) may be due to the increased lipid content of developing rats (Fig. 1). In fact, it was recently proposed that the levels of some molecules, like leptin, which are related to the adiposity of the body (Saladin et al. 1995), could act in the hypothalamus to reduce food intake (Schwartz et al. 1996). Measuring serum leptin levels, which were significantly increased in developing rats (Fig. 3), tested this hypothesis. Serum leptin levels correlated significantly with body fat mass and with daily ME intake/kg$^{0.75}$ (Fig. 3). Interestingly, in the last correlation, the slope of the regression line obtained for the period 90–180 d (-31.9 ± 7.4) was significantly ($P = 0.0068$) higher than that obtained for the period 25–90 d (-248.7 ± 10.2). This result suggests that from 90 d of age onward a progressive reduction in the responsiveness of the rat to the leptin signal with age takes place. Up to now, increased leptin resistance was only found in old rats (Li et al. 1997, Qian et al. 1998); our results suggest for the first time the possibility that an early onset of leptin resistance in developing rats is responsible for age-associated obesity.

The possibility that the reduction in daily energy expenditure, which occurs in developing rats, could be due to a decrease in basal metabolism has been a matter of debate (McCarter & Palmer 1992). However, to our knowledge, this question has not yet been addressed. To supply such information, we measured RMR in food-deprived rats at different ages (Fig. 2). RMR measured in this condition involves the energy cost of sustaining the body's vital functions, but not the thermic effect of food and locomotor activity, which are the other components of daily energy expenditure (Danforth 1992). Our results show that RMR significantly decreased from 25 to 55 d of age and remained constant thereafter (Fig. 2). On the other hand, daily energy expenditure decreased in rats with increasing age, reaching the lowest value in 90-d-old rats (Fig. 2). Thus, it can be suggested that the reduction in daily energy expenditure found in growing rats is achieved by a decrease in the thermic effect of food and/or locomotor activity. It should be noted that serum FT_3 levels correlated with RMR values between 60 and 180 d of age ($r^2 = 0.57$, $P < 0.05$), in agreement with the hypothesis that T_3 is the major hormonal determinant of RMR (Freake and Oppenheimer 1995). On the other hand, the discrepancy between RMR and serum FT_3 levels in the younger rats could be partly due to higher thermoregulatory needs of small animals at 24°C.

In conclusion, in the present work we found that the inability of adult rats to maintain a stable body weight cannot be attributed to a decrease in resting metabolism but rather to an early insensitivity to the satiating effect of leptin.

LITERATURE CITED

Barr, H. G. & McCracken, K. J. (1984) High efficiency of energy utilisation in "cafeteria" and force-fed rats kept at 29°C. Brit. J. Nutr. 51: 379–387.

Barzilai, N. & Rossetti, L. (1995) Relationship between changes in body composition and insulin responsiveness in models of the aging rat. Am. J. Physiol. (Endocrinol. Metab. 32) 269: E591–E597.

Danforth, E. J. (1992) Regulation of energy expenditure. In: Obesity: Basic concepts and clinical aspects, (Belfiore, F., Jeanrenaud, B. & Papalia, D. eds.) vol. 11, pp. 61–67. Basel, Karger.

Dulloo, A. G., Seydoux G. & Girardier L. (1990) Role of corticosterone in adaptive changes in energy expenditure during refeeding after low caloric intake. Am. J. Physiol. 259 (Endocrinol. Metab. 22): E658–E664.

Folch, J., Lees, N. & Stanley, G. H. S. (1957) A simple method for the isolation and purification of total lipids from animal tissues. J. Biol. Chem. 226: 497–510.

Freake, H. C. & Oppenheimer, J. H. (1995) Thermogenesis and thyroid function. Annu. Rev. Nutr. 15: 263–291.

Iossa, S., Mollica, M. P., Lionetti, L., Barletta, A. & Liverini, G. (1995) Hepatic mitochondrial respiration and transport of reducing equivalents in rats fed an energy dense diet. Int. J. Obes. 19: 539–543.

Iossa, S., Mollica, M. P., Lionetti, L., Barletta, A. & Liverini, G. (1997) Energy balance and liver respiratory activity in rats fed on an energy-dense diet. Br. J. Nutr. 77: 99–105.

Li, H., Matheny, M., Nicolson, M., Tumer, N. & Scarpace, P. J. (1997) Leptin gene expression increases with age independent of increasing adiposity in rats. Diabetes, vol. 46 (12): 2035–2039.

Lionetti, L., Iossa, S., Brand, M. D. & Liverini G. (1996a) The mechanism of stimulation of respiration in isolated hepatocytes from rats fed an energy-dense diet. Nutr. Biochem. 7: 571–576.

Lionetti, L., Iossa, S., Brand, M. D. & Liverini, G. (1996b) Relationship between membrane potential and respiration rate in isolated liver mitochondria from rats fed an energy dense diet. Mol. Cell. Biochem. 158: 133–138.

Liverini, G., Iossa, S. & Barletta, A. (1994) Hepatic mitochondrial respiratory capacity in hyperphagic rats. Nutr. Res. 11: 1671–1682.

Liverini, G., Iossa, S., Mollica, M. P., Lionetti, L. & Barletta, A. (1996) Hepatic fatty acid-supported respiration in rats fed an energy dense diet. Cell Biochem. Funct. 14: 283–289.

Masoro, J. E. (1980) Rats as a model for the study of obesity. Exp. Aging Res. 6: 261–270.

McCarter R. J. & Palmer J. (1992) Energy metabolism and aging: a lifelong study of Fisher 344 rats. Am. J. Physiol. 263 (Endocrinol. Metab. 26): E448–E452.

Newby, F. D., Digirolamo, M., Cotsonis, G. A. & Kutner, M. H. (1990) Model of spontaneous obesity in aging male Wistar rats. Am. J. Physiol. (Regulatory Integrative Comp. Physiol. 28) 259: R1117–R1125.

Pullar, J. D. & Webster, J. F. (1977) The energy cost of fat and protein deposition in the rat. Brit. J. Nutr. 37: 355–363.

Qian, H., Azain, M. J., Hartzell, D. L. & Baile, C. A. (1998) Increased leptin resistance as rats grow to maturity, Proc. Soc. Exp. Biol. Med. 219: 160–165.

Saladin, R., De Vos, P., Guerre-Millo, M., Leturque, A., Girard, J., Staels, B. & Auwerx, J. (1995) Transient increase in obese gene expression after food intake and insulin administration. Nature 377: 527–529.

Schwartz, M. W., Seeley, R. J., Campfield, L. A., Burn, P., & Baskin D. G. (1996) Identification of targets of leptin action in rat hypothalamus. J. Clin. Invest. 98 (5): 1101–1106.