Comparative Medicine
Copyright 2005
by the American Association for Laboratory Animal Science

Vol 55, No 1
February 2005
Pages 24-29

Meal-Feeding Studies in Mice: Effects of Diet on Blood Lipids and Energy Expenditure

Elizabeth Jane Parks, PhD,* Tara Lynn Schneider, and Rachel Ann Baar

To identify optimal study-design conditions to investigate lipid metabolism, male, C57BL/6J mice (age, 59 ± 3 days) were allotted to eight groups, with six animals per group that were stratified by three factors: diet type (high fat [HF]: 60% of energy from fat versus that of a standard rodent diet, 14% fat, fed for 7 weeks), feeding regimen (ad libitum [ad lib] versus meal fed), and metabolic state (data collected in fasted or fed states). Serum free fatty acids (FFA) and triacylglycerols (TAG) concentrations, and energy expenditure (EE) were assessed. Mice gained 0.30 ± 0.11 g of body weight/day when allowed ad lib access to HF diet, similar weight when meal-fed the HF or ad lib-fed the standard diet (0.10 ± 0.03 g/day), and no weight when meal-fed the standard diet (0.01 ± 0.02 g/day). Fed-state TAG concentration was 88 to 100% higher ($P < 0.02$) than that of the fasted state, except when animals were ad lib-fed the HF diet. When the standard diet was meal fed, FFA concentration was 30% higher in the fasted compared with the fed state ($P = 0.003$). Mice had 33% higher postprandial EE when either diet was meal fed ($P = 0.01$). Mice adapted to meal feeding developed transitions in metabolism consistent with known physiologic changes that occur from fasting to feeding. When fed the standard diet, a 6-h per day meal-feeding regimen was restrictive for normal growth. These data support use of a meal-feeding regimen when HF diets are used and research is focused on metabolic differences between fasted and fed states. This protocol allows study of the metabolic effects of an HF diet without the confounding effects of over-consumption of food and excess body weight gain.

Genetic strains of mice have provided powerful models for investigation of the regulation of lipid metabolism (2, 13, 16) and through their study, the molecular details of the genes impacting fatty acid synthesis, storage, and transport have been uncovered (12, 16, 26). As a result, additional strategies have been needed for the study of whole-body metabolism (5, 9, 10). However, the work of generating large numbers of transgenic mice is time consuming and the number of mice available for studies frequently is limited. Many mouse strains provide good models for the study of lipid metabolism, and these developments have fueled the expansion of translational research (11). Technical developments have begun to support investigation of the metabolic responses to genetic modifications to better define the phenotypes of genetically altered animals (1, 15, 21, 23, 28). At the same time these developments have been taking place, the importance of studying postprandial metabolism has become recognized (7, 17, 18, 20, 24).

Critical differences in lipid metabolism in the fasted and fed states underscore the need to understand the control of smooth transitions that occur between in vivo use of endogenous fuels during the fasting period and use of dietary components in the postprandial state. Indeed, key components of this regulation may be lost during development of disease states such as diabetes mellitus, and therefore, it is important to study differences in the metabolic control between fasting and feeding. Mice eat intermittently throughout the dark cycle and consume some food during the light cycle. Among the challenges of studying differences between fasted and fed states is that it is difficult to know exactly when animals are in these two distinct metabolic states. One solution to this problem is to train animals to eat all of their food in a given period, and the most notable use of such a "meal-feeding paradigm" has been used in investigations of the effect of nibbling versus gorging on body weight (3, 4, 27). The goal of the present study was to determine whether use of a meal-feeding regimen would maximize our ability to detect differences in metabolic parameters between the fasted and fed states. To accomplish this in an inexpensive manner, we chose the use of standardized cages easily available in any animal facility. A meal-feeding period of 6 h was used so that the activities associated with presenting and removing the food from the mice could be accomplished in a typical 8-h workday. When either a high-fat or the standard diet was meal fed, we found that essential differences in lipid metabolism in the fasted and fed states were evident in mice fed both diets. However, the meal-feeding protocol provided the greatest advantage when the high-fat diet was fed.

Materials and Methods

Animal husbandry and overall experimental design. Male C57BL/6J mice were purchased from The Jackson Laboratories (Bar Harbor, Maine); they arrived at the animal facility at 59 ± 3 days of age. Mice were housed in a barrier facility with a health monitoring program; all microbiological agents were excluded, and animals were free of rotavirus and mouse hepatitis virus. All procedures performed on mice were approved by the University of Minnesota Animal Care and Use Committee (Protocol No. 0106A04122) and satisfied the Research Animal Resources requirements for humane care and use of animals.

Mice were maintained on a 12-h reverse light/dark cycle (dark cycle was from 6 a.m. to 6 p.m.), given ad libitum access to water,

Received: 6/28/04. Revision requested: 8/2/04. Accepted: 8/3/04.
Department of Food Science and Nutrition, University of Minnesota—Twin Cities, 1334 Eckles Avenue, St. Paul, Minnesota 55108.
**Corresponding author.*

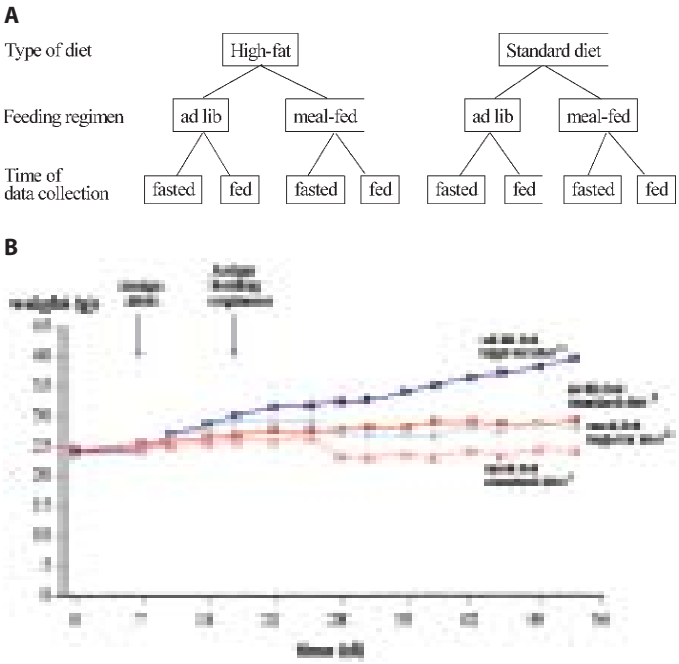

Figure 1. Scheme for randomization of the study (A) and body weight of mice (B). (A) Forty-eight male C57BL/6J mice were randomly assigned to groups differing in dietary intake, time of food consumption, and metabolic state when data were collected. (B) Time zero denotes the beginning of the study, when mice were 59 ± 3 days of age. Values are mean ± SD for groups (n = 12/group). At the day-52 time point, data with different superscript letters are significantly different, $P < 0.0001$.

and allowed to acclimate to the animal facility for 7 days while being fed a standard mouse diet (Product No. 8640, 22-5(W) rodent diet, Harland Teklad, Madison, Wis.) providing 23.6% of energy as protein (226 g/kg), 12.3% as fat (52 g/kg), and 64% as carbohydrate (612 g/kg). After this seven-day period, mice were randomized to one of two diets (Fig. 1A): the standard diet (continued on the diet described previously) or a high-fat diet (Product No. F3282, Bio-Serv, Frenchtown, N.J.) providing 15.2% of energy as protein (197.4 g/kg), 59.4% as fat (358.0 g/kg), and 24.5% as carbohydrate (358.2 g/kg).

Ten days after dietary assignment, mice in the two diet groups were further randomized to one of two feeding regimens: continued ad libitum (ad lib) intake or switched to a meal-feeding schedule of food availability for 6 h per day from 11:30 a.m. to 5:30 p.m. (in the dark cycle). To ensure meal-feeding status, mice were physically transferred to separate cages for the feeding and fasting phases of the day, with one of the two cages never containing food. This reduced the chance that mice hoarding food in the bedding would compromise the fasted state data. Meal- and ad lib-fed mice were also handled more than usual, which could offer potential benefit in reducing animal stress on the day of data collection. The four groups of mice (12 mice/group) were followed for 5.5 weeks to determine the effects of these regimens on food intake, body weight, and energy expenditure. Data were collected from half the mice of each group in the fasted state and from the other half in the fed state (Fig. 1A).

Indirect calorimetry and metabolite measurements. Throughout days 23 and 56 of the study, indirect calorimetry was performed in random order on individual mice by using a computer-controlled, open-circuit system (Applied Electrochemistry Inc., Pittsburgh, Pa.). A primary gas standard containing 20.0% oxygen, 1.0% carbon dioxide, and a nitrogen balance (National Specialty Gases, Research Triangle Park, N.C.) was used to calibrate carbon dioxide and oxygen sensors before each mouse entered the chamber for indirect calorimetry measurement. Mice were acclimated to the equipment during the five days before the measurements were made. To obtain data from fasted mice of the meal-feeding groups, measurements were taken from 8 to 11 a.m.—a time frame before which the animal would have (presumably) been anticipating food at 11:30 a.m. For the fed-state measurements, data were collected between 12 noon and 3 p.m.—at least 1 h after the animal had begun to eat. For animals eating ad libitum, fasted data were obtained after food had been removed at the beginning of the light cycle the night before the measurement (6:00 p.m.). Measurements were made between 8 and 11 a.m. the next day (after a 14-h fasting period during the light cycle). It is acknowledged that this protocol may have increased the stress in animals with previous accessibility to food at all times. For fed state, ad lib measurements, animals were observed to begin eating at 6 a.m. when lights went off, and the measurements were made between 12 noon and 3 p.m. (6 h after typical intermittent food intake). Values of oxygen consumption (milliliters per minute) and carbon dioxide production (milliliters per minute) were taken every 2 min for 3 h; all time points between 1 h and 2 h were inspected and data were averaged and used for calculation of respiratory quotient (RQ) and energy expenditure (EE) (14).

At the end of the study, animals were killed by lethal injection of a ketamine-xylazine (2:1) combination, and blood was obtained via open-heart puncture. Serum was immediately separated. Concentrations of serum free fatty acids (FFA; kit No. 994-75409 E, Wako Chemicals USA, Richmond, Va.) and triacylglycerols (TAG; kit No. 336, Sigma Diagnostics, St. Louis, Mo.) were measured enzymatically in triplicate, using a Microtek EL340 microplate reader (Bio-tek Instruments, Winooski, Vt.). The intra-assay co-efficient of variation was 7% for FFA and 8% for TAG.

Statistical analysis. Results are expressed as mean ± SD. Statistical differences between groups of fasted and fed mice were determined, using two-tailed, unpaired Student's t tests, with a value of $P < 0.05$ considered significant. Thereafter, control for multiple comparisons (analysis of variance for effects due to feeding regimens and diets) was imposed by reducing the P-value needed for significance. Data analysis was performed, using Microsoft Excel (2002 version, Microsoft Corporation, Redmond, Wash.), and statistical analyses were performed using Statview (version 5.0.1., SAS Institute Inc., Berkeley, Calif.).

Results

Body weight and metabolite concentrations. As expected, the high-fat (HF) diet resulted in significantly greater body weight gain when consumed ad lib compared with all other feeding regimens (Fig. 1B). From day 23 to day 56 of the study, the rate of weight gain in ad lib-, HF diet-fed mice was 0.30 ± 0.11 g/day, which was higher than the gain for meal-, HF diet-fed and ad lib-, standard diet-fed groups (0.10 ± 0.03 g/day). Body weight for the meal-, standard diet-fed animals remained constant (e.g., gain of 0.01 ± 0.02 g/day). For clarity when reporting the remainder of the outcome variables, data presentation will be uniform in first reporting differences between the fasted and

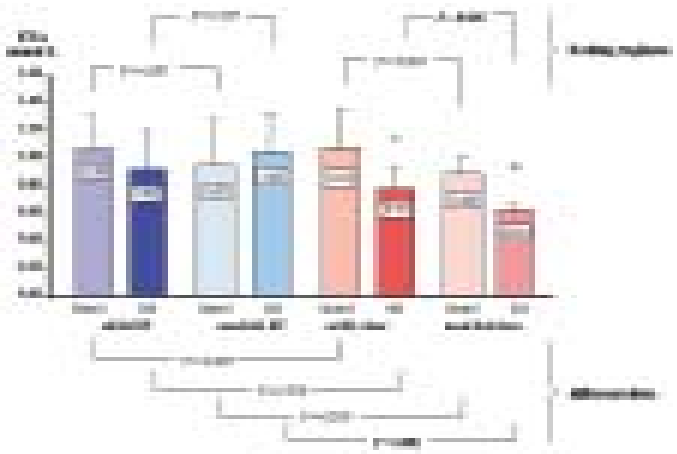

Figure 2. Serum free fatty acids (FFA) concentrations in the fasted and fed states. Serum FFA concentrations were measured in fasted (hatched bars) and fed (filled bars) mice. Asterisk denotes significant differences between nonfed and fed values within a single dietary treatment (n = 6/group). Also noted are *P*-values for comparisons of feeding regimen and dietary treatment. The absolute value of the mean is denoted on each bar. HF = High-fat diet.

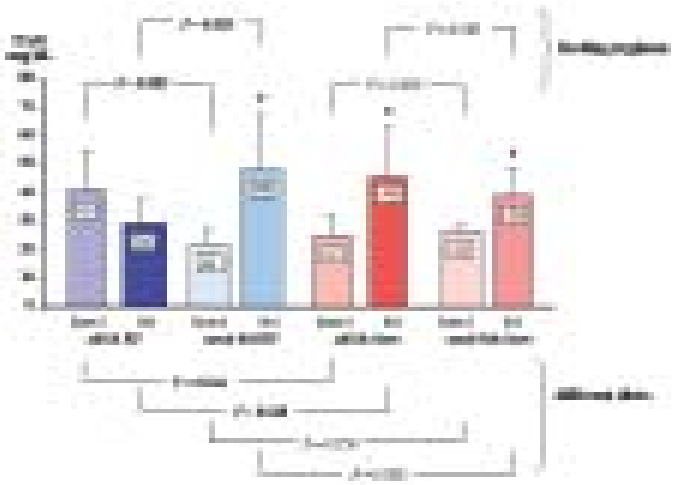

Figure 3. Serum triacylglycerol (TAG) concentrations in the fasted and fed states. Serum TAG concentrations were measured in fasted (hatched bars) and fed (filled bars) mice. *See* Fig. 2 for key.

fed states within a diet group (HF or standard diet) and feeding regimen (ad lib or meal fed). This information will be followed by presentation of differences between the feeding regimens within a particular diet. Lastly, comparisons will be presented based on differences between diet types. As indicated (Fig. 2), serum FFA concentration was significantly higher in the standard diet-fed mice in the fasted compared with the fed state, regardless of feeding regimen (significance denoted by asterisks). Between the two feeding regimens (*P*-value at the top of the graph), mice which were meal-fed the standard diet had lower FFA concentration in the fed state (0.79 ± 0.16 mmol/liter for ad lib-fed, standard diet versus 0.64 ± 0.03 mmol/liter for meal-fed, standard diet, $P = 0.006$). Furthermore, postprandial FFA concentration was significantly lower under meal-fed, standard diet conditions (0.64 ± 0.16 mmol/liter) compared with that under meal-fed, HF diet conditions (1.05 ± 0.23 mmol/liter, $P = 0.001$). By contrast, postprandial FFA concentration was not different between the diets when they were fed ad lib. A power calculation was performed comparing the postprandial values for ad lib diets to determine the number of animals that would have been needed to detect lower FFA concentration in the fed state. The result of this calculation was that 15 mice per group would have been required to detect a difference with 90% power ($P < 0.05$). Lastly, for the HF diet, FFA concentration was similar between fasted and fed states, regardless of feeding regimen.

Compared with fasted state values, serum TAG concentration was significantly higher in the fed state for all groups except the ad lib-, HF diet-fed animals (Fig. 3). By contrast, for the standard diet, different feeding regimens did not alter the pattern of higher postprandial TAG values. With respect to feeding regimen, meal feeding in animals of the HF diet-fed group resulted in significantly higher postprandial TAG concentration (48 ± 20 mg/dL versus 30 ± 8 mg/dL, $P = 0.033$), and fasting of animals of the HF diet-fed group resulted in significantly lower postprandial TAG concentration (22 ± 5 mg/dL versus 41 ± 14 mg/dL, $P = 0.005$). A significant interaction existed in the relationship between fasted and fed state TAG concentration and diet type. Specifically, in the fasted state, TAG concentration was higher in the ad lib-, HF diet-fed group than that in the ad lib-, standard diet-fed group ($P = 0.014$), whereas in the fed state, TAG concentration was lower in the ad lib-, HF diet-fed group than that in the ad lib-, standard diet-fed group, although the *P*-value for this comparison was only 0.048.

Energy expenditure and substrate oxidation. In mammals, the range of RQ values usually spans 0.70 and 1.00, with lower values indicating greater whole-body fat oxidation, and higher values (those close to 1.00) indicating greater glucose oxidation (14). In mice consuming typical diets, lower RQ would be expected in the fasted state, representing reliance of the body on adipose-derived fatty acids to provide energy when the animal is not eating. Higher RQ would be expected in the fed state for the standard diet-fed animals, exemplifying a postprandial switch in substrate utilization toward oxidation of dietary carbohydrate. In the study reported here, only the mice consuming the standard diet had a fasted state RQ that was lower than the fed state RQ, no matter which feeding regimen was used (Fig. 4).

Feeding did not increase RQ in HF diet-fed mice. With respect to the feeding regimen, meal feeding of the standard diet resulted in a significantly higher postprandial RQ (1.06 ± 0.09) compared with that for ad lib feeding of the standard diet (0.87 ± 0.06, $P = 0.001$). Similar to the FFA results, the only detectible effect of the different diets on RQ was associated with meal feeding of the diets. Meal feeding of the HF diet resulted in lower postprandial RQ (0.85 ± 0.09) compared with meal feeding of the standard diet (RQ = 1.06 ± 0.09, $P = 0.002$ for the difference).

In the postprandial state, EE associated with feeding is expected to be higher than that associated with fasting due to the thermic effect of food. However, as indicated (Fig. 5), the differences between fasted and fed state EE were only significant when associated with meal feeding. The increase in postprandial EE when the high-fat diet was meal fed resulted in a value (0.22 ± 0.04 cal/g/min) that tended to be higher than that for the HF diet fed ad lib (0.18 ± 0.04 cal/g/min, $P = 0.054$). Lastly, the effect of diet type (HF versus standard) on EE expenditure was evident when diets were fed ad lib. The standard diet fed ad lib was associated with higher EE than was the HF diet fed ad lib in the fasted ($P = 0.038$) and fed states ($P = 0.029$).

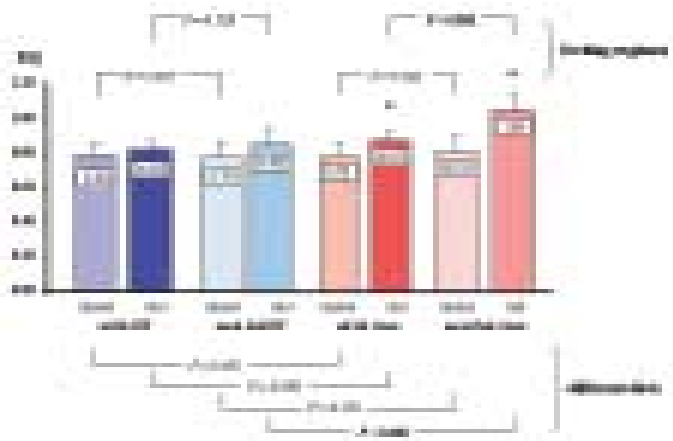

Figure 4. Respiratory (RQ) quotients from fasted and fed mice. Respiratory gas measurements were made in fasted (hatched bars) and fed (filled bars) mice. *See* Fig. 2 for key.

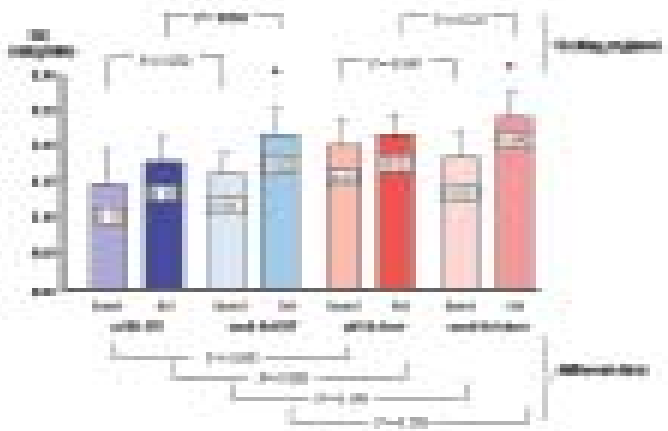

Figure 5. Energy expenditures (EE) in the fasted and fed states. The EE value was calculated from oxygen utilization in fasted (hatched bars) and fed (filled bars) mice. The absolute value of the mean is denoted on each bar, with units of energy expenditure in calories per gram of mouse body weight per minute. *See* Fig. 2 for key.

Discussion

Knowing when animals are fasted or fed. The goal of the study reported here was to determine whether switching mice to a meal-feeding regimen would maximize our ability to detect differences in metabolic parameters between the fasted and fed states. Of course, protocols for training animals to eat at specific times are not new (27), and some investigators have documented that the success of training an animal to meal-feed may depend on the time of the light cycle when the animal is given access to food (4, 6). Metabolic cages are available that have been fitted with electronic sensors to allow collection of precise information as to when and how much an animal has eaten. However, this equipment is expensive and the design of such cages does not always allow the animal to live in a comfortable environment with bedding. With use of standard animal cages, one common protocol to obtain fasted state data is to remove food from the cage 12 h before data collection. In an animal on a light cycle from 6 a.m. to 6 p.m., if food is removed at 6 p.m., the animal is denied food during the dark cycle. If this is the first time food has been unavailable to the mouse, data collected the next morning is likely to be confounded by stress (increasing FFA concentration). Such an animal has been in a fasted state for a minimum of 12 (dark) hours, but may have been in a fasted state longer than that, if the animal did not eat much the previous day in the light. Hence, the animal may be in a fasted state anywhere from 12 to 24 h, and the true extent of fasting will be unknown.

Some investigators use a reverse light-dark cycle (6 a.m. to 6 p.m. dark) and remove food at 6 p.m. (the beginning of the light cycle). The stress caused by the absence of food availability would still be present but have less of an impact since this protocol has the advantage of the food being denied during the light cycle (i.e., at a time when the animal would presumably not be eating much anyway). However, since animals eat most of their food at the beginning of the dark cycle, and do not eat continuously throughout the dark cycle, the exact duration of fasting is still unknown. Along the same line, animals fed ad lib consume food on and off during the 12-h dark cycle, and therefore, it is more difficult to know precisely when an animal is truly in the fed state. Regardless of the protocol used, the data presented here clearly indicate that differences in fasting protocols are important and the details of an animal's metabolic state at the time of data collection should be clearly stated in the methods section of reports.

Meal-feeding restrained body weight-gain in HF diet-fed animals. With this background in mind, the goal of our study was to determine whether switching mice to a meal-feeding regimen would maximize our ability to detect differences in metabolic parameters between the fasted and fed states. We wanted to accomplish this in an inexpensive manner so we chose the use of standardized cages available in any animal facility. We hypothesized that changes in substrate utilization expected as a result of feeding would be most readily detectible when animals were trained to meal feed. The main finding of this experiment was that under the HF feeding regimen, for all of the variables tested, switching an animal to meal feeding magnified the effects of fasting and feeding and allowed us to detect differences due to metabolic state with as few as six animals per group. By contrast, in standard diet-fed animals, fasting and fed state differences were detected in the ad lib- and meal-fed groups, and thus, meal feeding did not provide a significant advantage.

Meal feeding of the standard diet 6 h per day did not allow normal growth, although at the end of the 6-h feeding period, animals were resting quietly in their cages and did not appear to still be hungry. This leads us to conclude that, to maintain growth while consuming the standard diet, additional food intake must occur late in the dark cycle and sporadically in the light cycle. This conclusion is supported by the data generated through use of electronically monitored food cups, which indicated that mice typically consume approximately 70% of their daily energy in the dark cycle and 30% in the light cycle (23).

The aforementioned conclusions are based on the particular details of this study, which had a number of limitations. These limitations included that fact that the animals used for the studies were strain C57BL/6J; different strains of mice would likely respond differently to the constraints of meal feeding. Additionally, absolute food intake was not assessed. Food was presented on a metal grid above the cage because our goal was to mimic typical animal feeding protocols that might be used in studies not specifically focused on measuring food intake. Lastly, animals were physically transferred from a cage for fasting to a different cage for feeding. This handling may have increased the

stress on the mice, although, as described in the methods section, one of our goals was to allow animals to become accustomed to repeated human touch.

Effects of protocols on metabolic variables. Animals who were either meal-fed, or ad-lib fed the standard diet had fasted state serum FFA concentrations that were higher than fed state FFA concentrations. This would have been expected given the increase in adipose tissue fatty acid flux into the plasma in the fasted state. At that time, fasted animals in either standard diet group had lower RQ values compared with that of fed animals, which also was consistent with endogenous fatty acids providing most of the substrate for oxidation in the fasted state. Conversely, reductions in postprandial FFA concentrations of the standard diet-fed groups are consistent with higher insulin concentrations, which would inhibit adipose tissue hormone-sensitive lipase (8). Insulin concentration would be increased in animals actively eating the standard diet. The largest increase in postprandial RQ was found in meal-fed mice on the standard diet because an exact time could be reliably identified when the animal would be actively eating. This protocol resulted in the lowest variability of data for postprandial FFA concentration in animals meal-fed the standard diet. For the HF diet, FFA concentration was similar between the fasted and fed states, regardless of feeding regimen, which was most likely due to the HF nature of the diet. The FFA concentration in the fasted state would have been derived from adipose tissue lipolysis, whereas FFA concentration in the fed state would have been derived from fatty acids liberated by the action of capillary lipoprotein lipase and spillover into the plasma albumin pool (25). Thus, if the focus of future research studies were on differences in FFA metabolism between the fasted and fed states, meal feeding would not seem necessary. However, if the focus were on dietary effects (HF versus standard diet), meal feeding may provide some benefit.

For respiratory gas measurements, when the standard diet, ad lib- and meal-fed, was associated with RQ above 1.0 in the fed state, it indicated net postprandial de novo lipogenesis (14). Interestingly, fed-state postprandial TAG concentration also was increased, suggesting that lipogenesis was associated with increased plasma TAG concentration. Diet-induced differences in EE between the groups were small, except for a significantly lower EE in ad lib-, HF diet-fed animals compared with ad lib-, standard diet-fed animals. This lower EE associated with the high-fat diet was observed in the fasted and fed states. Thus, to compare the effect of a standard diet versus an HF diet on EE, meal feeding may not be necessary.

Summary and conclusions. In the study reported here, the value of the meal-feeding protocol became apparent when considering the effect of the HF diet on body weight, TAG metabolism, and EE. The benefits of this protocol are as follows. First, the HF diet-, meal-fed animals had similar weight gain as did animals fed the standard diet ad lib. Therefore, the meal-feeding regimen allowed investigation of the metabolic effects of HF diet without the confounding effects of over-consumption of food and excess body weight gain. In essence, the dietary effect could be isolated from effects associated with diet-induced obesity. Second, postprandial serum TAG concentration in the HF diet-fed groups would be expected to be high as a result of chylomicron production stimulated by the high quantity of dietary fat. This postprandial effect was not seen in ad lib-, HF diet-fed animals, but was clearly detectible in the HF diet-, meal-fed animals. Third, lack of difference in RQ between fasting and feeding states would have been expected and was observed in the HF diet-fed animals. When HF diets are consumed, postprandial substrate utilization would rely principally on dietary fatty acids, resulting in a fed-state RQ that should not differ from that of the fasted state, a time when use of endogenous fatty acids predominates. Fourth, meal-fed animals had significantly increased postprandial EE that averaged 35%. This magnitude most likely was a result of the combination of the thermic effect of food (expected to be 10 to 15%, [19]) and the physical activity associated with eating (grooming, movement to and from the feed cup), a concept that should be further investigated.

In conclusion, differences in lipid metabolism between fasted and fed states were exaggerated when an HF diet was fed, using a 6-h, meal-feeding regimen. The variability of data from ad lib-fed mice was larger and this increased the estimated number of animals that would be needed in the future to document differences, and thus, would also increase the time and expense of data collection. When genetically altered C57BL/6J mice are used in future studies designed to test the effect of an HF diet, a 6-h, meal-feeding regimen will successfully allow investigation of a particular gene manipulation without excess weight gain in the animals. One important benefit is that different effects of the genetics on fasted- and fed- state metabolism can be tested successfully using a limited number of mice.

Acknowledgments

This work was supported by an American Heart Association Scientist Development Grant to EJP, and in part, by a grant from the NIH-sponsored Minnesota Obesity Center (DK 50446-06).

References

1. **Alb, J. G., J. D. Cortese, S. E. Phillips, R. L. Albins, T. R. Nagy, B. A. Hamilton, and V. A. Bankaitis.** 2003. Mice lacking phosphatidylinositol transfer protein-a exhibit spinocerebellar degeneration, intestinal and hepatic steatosis, and hypoglycemia. J. Biol. Chem. **278:**33501-33518.
2. **Aouizerat, B. E., H. Allayee, J. Bodnar, K. L. Krass, L. Peltonen, T. W. de Bruin, J. I. Rotter, and A. J. Lusis.** 1999. Novel genes for familial combined hyperlipidemia. Curr. Opin. Lipidol. **10:**113-122.
3. **Baker, N. and R. J. Huebotter.** 1973. Lipogenic activation after nibbling and gorging in mice. J. Lipid Res. **14:**87-94.
4. **Baker, N., D. L. Palmquist, and D. B. Learn.** 1976. Equally rapid activation of lipogenesis in nibbling and gorging mice. J. Lipid Res. **17:**527-535.
5. **Bandsma, R. H., F. Stellaard, R. J. Vonk, G. T. Nagel, R. A. Neese, M. K. Hellerstein, and F. Kuipers.** 1998. Contribution of newly synthesized cholesterol to rat plasma and bile determined by mass isotopomer distribution analysis: bile-salt flux promotes secretion of newly synthesized cholesterol into bile. Biochem. J. **329:**699-703.
6. **Bazotte, R. B., M. Batista, and R. Curi.** 2000. Meal-feeding scheme: twenty years of research in Brazil. Braz. J. Med. Biol. Res**. 33**:985-991.
7. **Cohn, J. S.** 1998. Postprandial lipemia: emerging evidence for atherogenicity of remnant lipoproteins. Can. J. Cardiol. **14:**18B-27B.
8. **Frayn, K. N., S. W. Coppack, B. A. Fielding, and S. M. Humphreys.** 1995. Coordinated regulation of hormone-sensitive lipase and lipoprotein lipase in human adipose tissue in vivo: implications for the control of fat storage and fat mobilization. Adv. Enzyme Reg. **35:**163-178.

9. **Grefhorst, A., B. M. Elzinga, P. J. Voshol, T. Plosch, T. Kok, V. W. Bloks, F. H. van der Sluijs, L. M. Havekes, J. A. Romijn, H. J. Verkade, and F. Kuipers.** 2002. Stimulation of lipogenesis by pharmacological activation of the liver X receptor leads to production of large, triglyceride-rich very low density lipoprotein particles. J. Biol. Chem. **277:**34182-34190.
10. **Groen, A. K., V. W. Bloks, R. H. J. Bandsma, R. Ottenhoff, G. Chimini, and F. Kuipers.** 2001. Hepatobiliary cholesterol transport is not impaired in Abca1-null mice lacking HDL. J. Clin. Invest. **108:**843-850.
11. **Hall, J. E.** The promise of translational physiology. 2001. Am. J. Physiol. Gastrointest. Liver Physiol. **281:**G1127-G1128.
12. **Horton, J. D., J. L. Goldstein, and M. S. Brown.** 2002. SREBPs: activators of the complete program of cholesterol and fatty acid synthesis in the liver. J. Clin. Invest. **109:** 1125-1131.
13. **Hui, D. Y.** 1998. Utility and importance of gene knockout animals for nutritional and metabolic research. J. Nutr. **128:**2052-2057.
14. **Jequier, E., K. Acheson, and Y. Schutz.** 1987. Assessment of energy expenditure and fuel utilization in man. Annu. Rev. Nutr. **7:**187-208.
15. **Loten, E. G., A. Rabinovitch, and B. Jeanrenaud.** 1974. In vivo studies on lipogenesis in obese hyperglycaemic (ob/ob) mice: possible role of hyperinsulinaemia. Diabetologia **10:**45-52.
16. **Matsuda, M., B. S. Born, R. E. Hammer, Y. A. Moon, R. Komuro, J. D. Horton, J. L. Goldstein, M. S. Brown, and I. Shimonura.** 2001. SREBP cleavage-activating protein (SCAP) is required for increased lipid synthesis in liver induced by cholesterol deprivation and insulin elevation. Genes Dev. **15:**1206-1216.
17. **Parks, E. J.** 2001. Recent findings in the study of postprandial lipemia. Curr. Atheroscler. Rep. **3:**462-470.
18. **Participants, C.** Symposium of postprandial lipoprotein metabolism. Atherosclerosis **141:**S1-S113, 1998.
19. **Poehlman, E. T., and E. S. Horton.** 1999. Energy needs: assessment and requirements in humans, p. 95-104. *In* M. E. Shils, J. A. Olsen, M. Shike, and A. C. Ross (ed.), Modern nutrition in health and disease, 9th ed. Williams and Wilkins, Philadelphia.
20. **Roche, H. M., and M. J. Gibney.** 2000. The impact of postprandial lipemia in accelerating atherothrombosis. J. Cardiovasc. Risk **7:**317-324.
21. **Scheja, L., L. Makowski, K. T. Uysal, S. M. Wiesbrock, D. R. Shimshek, D. S. Meyers, M. Morgan, R. A. Parker, and G. S. Hotamisligil.** 1999. Altered insulin secretion associated with reduced lipolytic efficiency in aP2-/- mice. Diabetes **48:**1987-1994.
22. **Shimomura, I., M. Matsuda, R. E. Hammer, Y. Bashmakov, M. S. Brown, and J. L. Goldstein.** 2000. Decreased IRS-2 and increased SREBP-1C lead to mixed insulin resistance and sensitivity in livers of lipodystrophic and ob/ob mice. Mol. Cell **6:** 7-86.
23. **Seburn, J. (Jackson Laboratories).** Personal communication.
24. **Sniderman, A. D.** 2000. Postprandial hypertriglyceridemia(s): time to enlarge our pathophysiologic perspective. Eur. J. Clin. Invest. **30:**935-937.
25. **Teusink, B., P. J. Voshol, V. E. H. Kahlmans, P. C. N. Rensen, H. Pijl, J. A. Romijn, and L. M. Havekes.** 2003. Contribution of fatty acids released from lipolysis of plasma triglycerides to total plasma fatty acid flux and tissue-specific fatty acid uptake. Diabetes **52:**614-620.
26. **Uysal, K. T., L. Scheja, S. M. Wiesbrock, S. Bonner-Weir, and G. S. Hotamisligil.** 2000. Improved glucose and lipid metabolism in genetically obese mice lacking aP2. Endocrinology **141:**3388-3396.
27. **Wood, J. D. and J. T. Reid.** 1975. The influence of dietary fat on fat metabolism and body fat deposition in meal-feeding and nibbling rats. Br. J. Nutr. **34:**15-24.
28. **Young, S. G., C. M. Cham, R. E. Pitas, B. J. Burri, A. Connelly, L. Flynn, A. S. Pappu, J. S. Wong, R. L. Hamilton, and R. V. Farese, Jr.** 1995. A genetic model for absent chylomicron formation: mice producing apolipoprotein B in the liver, but not in the intestine. J. Clin. Invest. **96:**2932-2946.