Reduced Suppression of Plasma Saturated Fatty Acid Mobilization and Oxidation by Feeding in Lymphoma-bearing Mice

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ABSTRACT

Lymphoma-bearing mice have a circulating lipid-mobilizing factor, but increased plasma free fatty acid (FFA) turnover has not been demonstrable in earlier studies using postabsorptive tumor-bearing mice. We hypothesized that FFA mobilization in lymphoma-bearing mice is only elevated in fed mice and may best be observed at night (dark, reversed light cycle). AKR mice with early and advanced tumors (10^6 SL-3 lymphoma cells, i.p.) and controls were fed ad libitum (reversed light cycle, dark) or fasted 4 h (daylight, regular cycle), given injections of [14C]bicarbonate or [1-14C]palmitate-mouse serum albumin, i.v., and plasma [14C]FFA disappearance and/or breath 14CO2 were monitored. Plasma FFA mobilization, estimated by multicompartamental analysis (SAAM) of the oxidation rate was lower in fasted mice with advanced tumors [tumor, 9.5 ± 6.0% (%SE); controls, 14 ± 4.4% μg-atoms fatty acid/min/30 g body weight, n = 3 to 6 mice/time point/group]. Feeding reduced these rates 90% in control mice and 53% in mice with early tumors, but only 14% in mice with advanced tumors. Plasma FFA fractional catabolic rates were 2.5 times faster in fed mice with advanced tumors than in controls. Diminished suppression of fatty acid mobilization in fed tumor-bearing mice (at night) probably accounts partially for the body fat loss.

INTRODUCTION

The loss of body fat seen in cancer-bearing rodents has been attributed, in part, to the presence of circulating LMF derived from the cancer cell (1-4). Lymphoma-bearing AKR mice, which lose most of their body fat during tumor growth, have been reported to have such a circulating peptide (1, 5). They may also have other cachexia-inducing peptides such as TNF/cachectin derived from the immune system (6), although this has not been studied in these mice, to our knowledge. Therefore, one would expect the mobilization and oxidation of plasma FFA to be greatly accelerated in lymphoma-bearing mice (1). However, the flux of plasma FFA, using [1-14C]palmitate complexed to mouse serum albumin as the tracer, has recently been reported to be somewhat reduced, rather than elevated (7), in direct contradiction to the hypotheses based upon the presence of LMF and TNF/cachectin in TB mice, and of LMF specifically in lymphoma-bearing mice. Clearly, one would not expect the loss of body fat in these mice to be accompanied by reduced FFA mobilization. However, a number of workers, including one of the present authors, have reported defects in lipogenesis (8), very low density lipoprotein-triaclyglycerol fatty acid removal from plasma (9) or lipoprotein lipase activity (10, 11), and FFA esterification in adipose tissue (12) of tumor-bearing mice. All of these could lead to loss of body fat in the absence of elevated rates of plasma FFA release.

Nevertheless, before rejecting the possibility that part of the body fat loss in cachectic mice results from increased fatty acid mobilization, we have explored another hypothesis. Although it seems clear from our earlier studies both in lymphoma-bearing (7) and EAT-bearing mice (13) that plasma FFA mobilization is not increased at advanced stages of tumor growth, when measured daytime in the briefly fasted state, there could be a defect in this insulin-dependent regulatory mechanism at night, when the animals consume a major portion of their daily food intake and when plasma FFA production from adipose tissue is normally reduced sharply (13-15, and Refs. cited therein). Such a regulatory defect has been suggested to exist in cancer-bearing human subjects (16).

In the present study we have used a recently described quantitative tracer approach (17) to estimate both plasma FFA turnover and oxidation rates. We have measured these fluxes in control and lymphoma-bearing mice (during both early and advanced stages of tumor growth) in two dietary states: fed ad libitum but briefly fasted on the morning of the study (regular light cycle) and fed ad libitum, studied in the dark (fed state, reversed light cycle). Control mice showed the expected large inhibition of plasma FFA mobilization and oxidation rates when studied in the fed compared to the briefly fasted state. However, the lymphoma-bearing mice at late stages of tumor growth continued to mobilize and oxidize plasma FFA [tracer, [1-14C]palmitate complexed to mouse serum albumin] in the fed state at about the same rate as in the fasted condition. The continued release of FFA from adipose tissue and the diminished suppression of plasma FFA oxidation during the night could account to a large extent for the loss of body fat during cancer growth in these mice.

MATERIALS AND METHODS

Animals and Tumors

Seven-week-old male AKR/J mice (The Jackson Laboratory, Bar Harbor, ME) were maintained on Purina laboratory chow and water ad libitum. The mice were divided into lymphoma-bearing and control groups. Mice in the TB group were inoculated i.p. with 1 x 10^6 freshly harvested SL-3 tumor cells that had been maintained in culture (18). The cells were injected i.p. in 0.2 ml medium or phosphate buffer (7). The tumor usually developed in about 2 weeks and was detected by a palpable abdominal mass near the inoculation site and by a decreased food intake.

The TB mice were studied at two "stages" of tumor growth (i.e., degrees of tumor burden), early and late. Solid tumors were dissected from the peritoneal cavity at autopsy, and tumor wet weights were measured. In mice with large tumor burdens, there was extensive infiltration of the tumor into most of the organs in the abdominal region. No attempt was made to quantify the extent of metastases. The right inguinal fat pads of both control and TB mice were also dissected at autopsy and weighed. Mean tissue and body weights are given in "Results." Food intake was measured by providing each mouse in an individual cage an excess, fixed amount (about 8 g) of Purina chow pellets and recording the weight of the remaining food the next morning. Spillage
of the food particles into the shavings inside the cage was considered negligible and was not taken into account.

In order to test the hypothesis that plasma FFA (palmitate) oxidation was increased in TB mice only at night, which is when both the TB and control animals consume most of their food, two types of light cycles were used. This allowed us to carry out the experiments in the early afternoon by using either briefly fasted (4–6 h) mice, studied in the light, or mice fed (ad libitum) and studied in the near dark. The normal light cycle consisted of 12 h of light (8 a.m.–8 p.m.) and 12 h of darkness at night. The reversed light cycle consisted of 12 h of darkness (8 a.m.–8 p.m.) followed by 12 h of light at night. Both the control and TB mice used under reversed light cycle conditions were placed under those conditions as soon as the tumor was inoculated and were maintained under those conditions throughout the tracer study, but with additional minimal lighting required to inject the tracer and to execute the experiment.

Experimental Protocols

Studies were carried out on separate populations of mice (rather than from a single randomized population subdivided into the appropriate subgroups and studied simultaneously, a more ideal experimental design). A brief summary of the experiments and, in parentheses, the number of mice used in each follows.

Experiment 1. Measurement of breath 14CO2 excretion in briefly fasted control (N = 8) and TB (N = 4); “late stage” (i.e., large tumor burden) mice, unanesthetized, regular light cycle, in the p.m. (light), after i.v. tracer injection of [1-14C]bicarbonate. The control data, which included both mice fed ad libitum and mice pair fed with the TB group, have been presented as part of an earlier study dealing with the validation of the model (17) and are shown again for comparison with the TB group. (The latter studies were carried out in collaboration with the late Dr. James Mead; see "Acknowledgements").

Experiment 2. Oxidation of plasma FFA to CO2 in control and TB (large tumor burden) mice, conditions as in experiment 1 (i.e., briefly fasted, regular light cycle, studied in the light) and also carried out in collaboration with Dr. Mead. Separate groups of unanesthetized mice were used to measure breath 14CO2 in controls (N = 6) and in TB (N = 6) mice and to measure plasma [14C]FFA disappearance in controls (N = 3/time point) and in TB (N = 3/time point) mice after tracer injection of [1-14C]palmitate i.v. Approximately one-half of the mice that were used in experiment 2, either for 14CO2 breath measurements or for FFA tracer disappearance studies, were anesthetized lightly with diethyl ether just before drawing the terminal blood samples at 10 min. Use of anesthesia in this way did not influence the tracer kinetic data. In this, as in all experiments involving blood sampling, only two serial blood samples (e.g., either at 0.5 and 2 min or at 1.0 and 5 min) were withdrawn prior to the terminal sample to minimize stress and effects of blood loss.

Experiment 3. Control mice, normal animals placed on a reverse light cycle, studied in the fed state, midday, near dark, and given injections of tracer [1-14C]palmitate i.v. on 2 consecutive days. Day 1, breath 14CO2 only, measured in unanesthetized mice (N = 6). Day 2, same animals, unanesthetized, used to measure plasma [14C]FFA disappearance, 2 time points/mouse, plus the terminal sample at 10 min (N = 3 at each of two times preceding the terminal sample).

Experiment 4. TB mice with small tumor burdens, same experimental design as in experiment 3 (i.e., reversed light cycle, fed state, midday, near dark, same tracer), but with N = 5 instead of 6. Of the 5 mice studied, 2 were excluded, one, because it ate no food on the day before the experiment, and the other because it had a very large tumor burden at autopsy and behaved metabolically as did the TB mice with large burdens. Therefore, N = 3 for both curves generated, i.e., 14CO2 and [14C]FFA, with only 2 mice used to obtain values for plasma [14C]FFA at the two earliest times.

Experiment 5. TB mice with large tumor burdens (N = 6), same conditions as in Experiments 3 and 4, but no plasma [14C]FFA data collected except for 2 terminal samples taken from mice that had been briefly anesthetized with Metofane (Pitman-Moore, Inc., Washington Crossing, NJ) immediately after the last breath 14CO2 sample was collected. The plasma radioactivity remaining at 11 min was the same (2.0%) as that found in Experiment 4; therefore, to minimize trauma and killing of mice and because sensitivity analyses showed that small differences in plasma FFA kinetics have little impact upon calculated rates of plasma FFA oxidation to CO2, the plasma FFA disappearance curve for the late TB mice was assumed to be the same as that of mice with small burdens (experiment 4).

Tracers

NaH14CO3 (7.7 mCi/mmol) was purchased from New England Nuclear, Wilmington, DE. It was diluted with slightly alkaline distilled water and injected (1 µCi/mouse in 0.2 ml) i.v. into the tail vein. [1-14C]Palmitic acid (58 mCi/mmol) was purchased from ICN Biomedicals, Inc. (Costa Mesa, CA). Fatty acid-mouse serum albumin complexes were prepared as described previously (19). The dose (1 µCi/mouse) in 0.2 ml mouse serum was injected into the tail vein of unanesthetized mice in restraining cages.

Blood Sampling

Blood was obtained from an ophthalmic venous sinus in heparinized capillaries. Only 2 blood samples/mouse (100 µl total volume) were taken prior to a third terminal sample. The capillaries were kept in ice and then centrifuged at 1000 rpm for 1 min to separate plasma from RBC.

Chemical and Radioactive Analyses

Plasma [14C]FFA was determined by adding 25–100 µl plasma to 10 ml Aquasol and counting directly, without prior separation of FFA from other lipids. We have confirmed in trial runs that the serum total neutral lipid radioactivity in the short time span (10 min) used in these experiments is entirely in the form of labeled FFA. Plasma FFA concentrations were determined by using terminal blood samples according to the method of Hron and Menahan (20) as in our earlier studies (17). Total plasma [14C]FFA radioactivity at each time was calculated by assuming a plasma volume of 4.0% of body weight (13).

Breath CO2 Measurement

The techniques used for measuring radioactivity and mass of CO2 were those described in our earlier study (17), namely, trapping in Hyamine hydroxide solution, measuring the time needed for the expired CO2 to titrate the latter to a phenolphthalein endpoint (21), and measurement of bicarbonate radioactivity by liquid scintillation counting (0.5 ml of the trapping agent in 10 ml Dimilume-30 (Packard Instrument Co.)]. Breath 14CO2 was collected for 10 min, with measurements of radioactivity every 2 min, cumulatively.

Multicompartmental Analyses

Multicompartmental analyses were carried out with the SAAM and CONSAM programs (22, 23) with a VAX 11-780 computer and the relevant portions of the model shown in Fig. 1, as described in earlier studies (7, 17). The symbols used in SAAM/CONSAM and referred to

Fig. 1. Model used to analyze plasma FFA turnover and oxidation rates in control and tumor-bearing mice. Compartments 1 and 2, plasma and extraplasmatic FFA, respectively; compartment 3, slowly mixing FA compartment(s) ["FA-X" (17)] that exchange FA with plasma FFA (13); broken arrow from compartment 3 to compartment 1 indicates that L(1, 3) is indeterminate, and the model could be fit to the data obtained during the first 10 min following tracer [1-14C]palmitate injection i.v. whether L(1, 3) was included or excluded; compartment 10, rapidly turning over intermediates involved in plasma FFA oxidation to CO2, compartments 6 and 7, plasma and extraplasmatic bicarbonate-CO2 compartments, respectively; compartment 8, breath CO2.

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compartment J; for example, $L(IO, 1)$ is the fractional rate constant (units, min$^{-1}$) into compartment 10 from compartment 1, where $I = 10$ and $J = 1$, as shown in Fig. 1. $R(I, J)$ is the transport rate into compartment 1 from compartment J with units of $\mu$g-atoms carbon/min/30 g body weight; thus, $R(IO, 10)$ refers to the transfer of material into compartment 10 from compartment 1. Finally, $U(J)$ is the rate of input of carbon into compartment J from "outside the system" [same units as $R(I, J)$]. For example, where $J = 1$, $U(1)$ is the flow into compartment 1 from outside the system. The logic and assumptions used in developing and validating the model have been presented in detail (17) and will not be reiterated here. The present analysis and model ignore delayed recycling (17), which would require a much more extended study to evaluate, and we have not attempted to define the size or kinetic behavior of the slowly exchanging FA compartment 3 (Fig. 1); emphasis was solely upon the estimation of the oxidation rate constant, $L(IO, 1)$, and the flux, $R(IO, 1)$, respectively. The latter was assumed to approximate $U(1)$, the rate of net FFA input into the system (17). The hypothesis being tested is concerned specifically with these rates. Nevertheless, despite the use of this simplified experimental design and model, the SAAM/CONSAM analysis also provided additional detailed information about the relative masses of the exchangeable compartments, M(2) and M(3).

Statistical Analysis

The significance of the differences between mean values (control versus TB mice for any fractional rate constants, or transport rates) was assessed by constructing an approximate 95% confidence interval about the estimated variance of the difference

$$D \pm 2 \sqrt{\hat{V}(D)},$$

where the estimated variance of the difference, $\hat{V}(D)$, was computed as the sum of the variances of the mean values. The latter follows from the fact that the means of the two groups being compared are, statistically, independent quantities. We have assumed that the means are normally distributed estimates. However, the SAAM/CONSAM instructions and the program itself warn the user that the fractional standard deviations generated may not be valid and that there are still unresolved aspects of the statistical evaluation of nonlinear kinetic data. Therefore, the statistical evaluation is only intended to serve as a basis for forming a tentative evaluation of the differences between the estimates obtained in each group. Differences between means of pool sizes were evaluated by using Student’s t test.

RESULTS

Mean Body Weights, Food Intake, Inguinal Fat Pad, and Tumor Wet Weights in Control and Lymphoma-bearing Mice. Table 1 lists the mean body weights, food intakes, and tissue weights of the mice used in this study. The body weights of TB mice with either small or large tumor burdens did not differ significantly from those of controls (Table 1). The early tumor-bearing and control mice had similar food intakes (3.5 and 3.9, respectively), while mice with larger tumors ate significantly less food than did the controls (2.5 ± 0.2 g versus 3.9 ± 0.3 g; $P < 0.01$). The inguinal fat pad wet weights in controls and in mice with small tumors were similar, but were apparently lower in mice bearing large tumors (49 ± 19 as compared to control mice, 138 ± 29 mg). The difference of means was not statistically significant. Late stage tumor-bearing mice had an average tumor mass of 3.4 g while the tumor mass in the early tumor-bearing group was ≤0.5 g.

Appearance of $^{14}$CO$_2$ in Breath following Injection of Labeled Bicarbonate in Lymphoma-bearing and in Control Mice: Modeling of the Bicarbonate-CO$_2$ System. Labeled CO$_2$ reached a maximum specific activity within the first 2 min following tracer [$^{14}$C]bicarbonate injection i.v. and then fell in a complex exponential manner in both control and TB mice. This can be seen from Fig. 2 which depicts the rate of $^{14}$CO$_2$ expiration (percentage of dose per min), a function that is directly proportional to the specific activity-time curve (not shown). Initially the rate of $^{14}$CO$_2$ expiration was greater in the controls than in the TB mice; however, within 10 min there was a reversal in this pattern. The mean rate of $^{14}$CO$_2$ expiration (mass/time) tended to be lower in the TB mice than in the controls [Table 2, R(0, 6)]. The corresponding fractional rate of CO$_2$ expiration [$L(0, 6)$] was significantly depressed ($P \leq 0.05$) in TB mice, from 0.33 ± 3.5% to 0.23 ± 6.4% per min. Since $^{14}$CO$_2$ specific activity is inversely proportional to the CO$_2$ expiration rate, i.e., specific activity = percentage of dose per min/$R(0, 6)$, the relative specific activity of breath $^{14}$CO$_2$ of tumor-bearing mice was also greater, even more so than suggested by the $^{14}$CO$_2$ excretion data (percentage of dose per min) of Fig. 2, than that of the control mice during most of the study.

The data shown in Fig. 2, coupled with the measured rates of expired CO$_2$ excretion, were analyzed by CONSAM/SAAM using a simple 2- or 3-compartment model. The results are shown in Table 2. Although the bicarbonate compartments (M6 and M7) tended to be somewhat enlarged in the tumor-bearing mice, and the flux of bicarbonate between the compartments faster than in the controls, 31 versus 21 $\mu$g-atoms CO$_2$-carbon/min/30 g body weight, the differences were not statistically significant. A similar trend was observed in an earlier study of EAT-bearing and control mice (24). As in previous studies (7, 17), the plasma bicarbonate kinetics were used primarily to define the kinetic behavior of newly formed [$^{14}$C]bicarbonate following the injection of labeled palmitate. Thus, the parameters shown in Table 2 were used as fixed values in the subsequent analyses of plasma palmitate oxidation to CO$_2$.

Plasma FFA Replacement and Oxidation Rates in Briefly Fasted Control and Lymphoma-bearing Mice. As noted in an earlier study, which was based on [$^{14}$C]palmitate disappearance from plasma studied over an extended time period (7), the plasma FFA irreversible disposal rate (assumed to approximate that of plasma FFA oxidation to breath CO$_2$) tended to be slower in lymphoma-bearing mice than in controls when the mice were studied in the briefly fasted state (regular light cycle, daylight, fasted approximately 4 h). This is evident from the data in Fig. 3 which shows that the mean percentage of dose excreted as $^{14}$CO$_2$ in 10 min in the tumor-bearing mice was reduced about 30% compared to controls. The compartmental analysis based upon the latter data and used to calculate plasma FFA replacement and oxidation rates is summarized in Table 3, discussed below, along with the statistical treatment of the differences. Clearly, there was no evidence of increased plasma

<table>
<thead>
<tr>
<th>Body weight*</th>
<th>Food intake</th>
<th>Inguinal fat pad</th>
<th>Tumor mass</th>
</tr>
</thead>
<tbody>
<tr>
<td>(g)</td>
<td>(g)</td>
<td>(mg wet wt)</td>
<td>(g)</td>
</tr>
<tr>
<td>Control</td>
<td>27.2 ± 1.3</td>
<td>3.9 ± 0.2</td>
<td>138 ± 29</td>
</tr>
<tr>
<td>TB (early)</td>
<td>24.5 ± 0.6</td>
<td>3.5 ± 0.6</td>
<td>150 ± 19</td>
</tr>
<tr>
<td>TB (late)</td>
<td>26.9 ± 1.1</td>
<td>2.5 ± 0.2</td>
<td>49 ± 19</td>
</tr>
</tbody>
</table>

* Reverse light cycle fed state. The body weights of control and late TB mice used in other experiments (regular light cycle, briefly fasted state) were 33.0 ± 1.1 g and 31.7 ± 0.8 g, respectively; mean ± SE (n = 3–4 mice/group).

* Less than 0.5 g of solid tumor was isolated in most mice. Formation of gelatinous ascites material but not quantified.

* NS, not significant; statistical analysis: late TB versus control.

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PLASMA FFA TURNOVER AND OXIDATION IN LYMPHOMA-BEARING MICE

Fig. 2. Excretion of $^{14}$CO$_2$ in breath after tracer injection of labeled bicarbonate in briefly fasted lymphoma-bearing and control mice. Tracer $[^{14}$C]bicarbonate was injected i.v. at zero time and the rate of labeled CO$_2$ excretion (percentage of injected dose/min) measured continuously over discrete intervals. Average $^{14}$CO$_2$ excretion rates are plotted at the midpoints of the intervals. The control data have been reported previously as part of an earlier study in which the model used here was validated in mice without tumors (17). Data in that study for ad libitum-fed controls and control mice pair-fed with the tumor group did not differ significantly and were combined. Bars, SE; number of mice, 8 (combined controls) and 4 (lymphoma-bearing). The curves shown are the least square fits obtained by CONSAM/SAAM analyses of the data using a 2-compartment model corresponding to compartments 6 and 7 in Fig. 1. See Table 2 for the calculated fractional rate constants, transport rates, and pool sizes.

Reduced Suppression of Plasma FFA Replacement and Oxidation Rates in Fed (Compared to Briefly Fasted) Lymphoma-Bearing Mice. The well-known inhibition of plasma FFA mobilization and oxidation by eating was readily demonstrated in control mice, as shown in Fig. 4 (rates summarized in Table 3, discussed below). Thus, both the early disappearance of plasma $[^{14}$C]FFA and the appearance of its major oxidation product, $^{14}$CO$_2$, were depressed markedly in the fed state (reversed light cycle, daytime, dark) compared to the briefly fasted state (Fig. 5). Only one-third as much radioactivity appeared cumulatively in the breath in 10 min in the fed compared to the briefly fasted state (7.3 ± 2.0 versus 23 ± 1.5% dose), $P < 0.01$.

In contrast, however, there was no inhibition of FFA mobilization and oxidation in fed (reversed light cycle, dark) lymphoma-bearing mice with large tumor burdens (Fig. 5). Plasma FFA oxidation was inhibited in mice with small tumor burdens, but less so than in control mice, as shown in Fig. 5.

The results of our multicompartmental analysis of these data are shown in Table 3. Looking first at the plasma FFA pool sizes, there was a significant decrease in plasma FFA in the fed compared to the briefly fasted mice in the controls (from 15 to 8.8 µ-atoms FA-carbon/30 g body weight). No decrease was

Table 2 Fractional rate constants, transport rates, and pool sizes defining the bicarbonate-CO$_2$ system in lymphoma-bearing and control mice

<table>
<thead>
<tr>
<th>Pool sizes (µ-atoms bicarbonate-CO$_2$-carbon/30 g body wt ±%SE)*</th>
<th>Combined controls</th>
<th>Lymphoma-bearing</th>
</tr>
</thead>
<tbody>
<tr>
<td>M(6)</td>
<td>280 ± 4.8</td>
<td>310 ± 6.9</td>
</tr>
<tr>
<td>M(7)</td>
<td>300 ± 11</td>
<td>370 ± 19</td>
</tr>
<tr>
<td>Fractional rate constants/min ±%SE</td>
<td></td>
<td></td>
</tr>
<tr>
<td>L(6, 7)</td>
<td>0.069 ± 7.6</td>
<td>0.083 ± 11</td>
</tr>
<tr>
<td>L(7, 6)</td>
<td>0.074 ± 11</td>
<td>0.10 ± 13</td>
</tr>
<tr>
<td>L(0, 6)</td>
<td>0.33 ± 3.5</td>
<td>0.23 ± 6.4*</td>
</tr>
<tr>
<td>Transport rates (µ-atoms CO$_2$-carbon/min/30 g body wt ±%SE)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>R(6, 7)</td>
<td>21 ± 8.9</td>
<td>31 ± 16</td>
</tr>
<tr>
<td>R(7, 6)</td>
<td>21 ± 8.9</td>
<td>31 ± 16</td>
</tr>
<tr>
<td>R(0, 6)</td>
<td>94 ± 3.3 (n = 23)</td>
<td>72 ± 6.7 (n = 18)</td>
</tr>
<tr>
<td>U(6)</td>
<td>94 ± 3.3</td>
<td>72 ± 6.7</td>
</tr>
</tbody>
</table>

* Control values were published previously in a theoretical paper (17); data for both controls and TB mice were obtained in the present controlled study.

Pool sizes and SE values were calculated from steady state relations based upon the measured rates of CO$_2$-C excretion in the breath $R(0, 6)$, their SEM values, the fractional rate constants and their %SE (%SD in SAAM notation) as generated by SAAM analyses of the data shown in Fig. 1. The model used has been described earlier (17) and corresponds to the last 3 compartments (6-8) shown in Fig. 1, where compartment 8 = breath CO$_2$; i.e., $R(0, 6) = R(8, 6)$.

Statistical analysis: no significant differences, TB versus controls, for any parameter except L(0, 6), where $P < 0.05$. 

Fig. 3. Disappearance of plasma $[^{14}$C]FFA (A) and appearance of breath $^{14}$CO$_2$ (B) after i.v. injection of [1-$^{14}$C]palmitate complexed to mouse serum albumin in lymphoma-bearing (x) and control (•) mice. The animals were previously fed ad libitum, during the day and maintained on a regular light cycle. They were fasted 4–5 h; experiments were carried out in the early p.m. in the light. Ordinate units in both A and B are percentage of injected dose. In A, the data are plotted semilogarithmically in a but linearly in B; bars, SE. Number of mice: A, 3/time point, except 6 terminal; B, 6.
Table 3  Plasma FFA pool sizes and rates of replacement and oxidation to CO2 in briefly fasted and in fed control and lymphoma-bearing mice

<table>
<thead>
<tr>
<th>Group</th>
<th>Pool sizes [M(I)]</th>
<th>Fractional rate constants [L(10, 1)]</th>
<th>Oxidation rates [R(10, 1)]^a</th>
</tr>
</thead>
<tbody>
<tr>
<td>Controls</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Briefly fasted</td>
<td>15 ± 2.7^a</td>
<td>0.96 ± 3.5</td>
<td>14 ± 4.4</td>
</tr>
<tr>
<td>Fed</td>
<td>8.8 ± 4.9</td>
<td>0.16 ± 4.0</td>
<td>1.4 ± 6.3</td>
</tr>
<tr>
<td>Early tumor</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Briefly fasted</td>
<td>15</td>
<td>0.80</td>
<td>12</td>
</tr>
<tr>
<td>Fed</td>
<td>19 ± 8.4</td>
<td>0.30 ± 11</td>
<td>5.7 ± 14</td>
</tr>
<tr>
<td>Late tumor</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Briefly fasted</td>
<td>15 ± 2.7^a</td>
<td>0.63 ± 5.4</td>
<td>9.5 ± 6.0</td>
</tr>
<tr>
<td>Fed</td>
<td>19 ± 8.4^a</td>
<td>0.43 ± 5.6</td>
<td>8.2 ± 10</td>
</tr>
</tbody>
</table>

^a Plasma FFA oxidation rates are assumed to approximate the replacement rates.

Statistical analysis for R(10, 1): briefly fasted controls versus fed controls, P ≤ 0.01. Fed early tumor versus fed controls, P ≤ 0.05. Fed late tumor versus fed controls, P ≤ 0.01. Briefly fasted late tumor versus fed late tumor, not significant.

Based on a separate study of lightly anesthetized control and tumor-bearing mice from which only a single orbital blood sample was withdrawn; values of control and TB mice did not differ significantly. The mean value for combined unanesthetized control and TB mice from which serial orbital blood samples were obtained was 20 ± 6.0%.

Not studied; assumed average of other briefly fasted (control and late tumor) values.

Pool sizes not measured; assumed equal to those of early TB group in fed state.

Fig. 4. Disappearance of plasma [14C|FFA (A) and appearance of breath [14CO2 (B) after i.v. injection of [1-14C]palmitate complexed to mouse serum albumin in briefly fasted (x) and fed lymphoma-bearing mice (Δ, early tumors; □, late tumors). Experiments were done in the day (regular light cycle for the briefly fasted mice; reversed light cycle, dark, for the fed mice). Units and other details as in Fig. 3. The data for the briefly fasted lymphoma-bearing mice (late tumors) are the same as those shown in Fig. 3 (x). Number of mice for the fed TB group: A, 2-3/time point, 3 terminal; B, 3 and 6 for the early and late TB groups, respectively. The data for the fed late TB mice are the same as those shown in Fig. 3 (x). Number of mice for the fed late TB group: A, 2-3/time point, 3 terminal; B, 3 and 6 for the early and late TB groups, respectively. FFA disappearance curves were not obtained for the fed late TB mice (assumed for SAAM/CONSAM analysis, not to differ from the fed early-mid TB group; see "Experimental Protocols, Experiment 5" for additional data supporting this assumption).

Fig. 5. Disappearance of plasma [14C|FFA (A) and appearance of breath [14CO2 (B) after i.v. injection of [1-14C]palmitate complexed to mouse serum albumin in briefly fasted (x) and fed lymphoma-bearing mice (Δ, early tumors; □, late tumors). Experiments were done in the day (regular light cycle for the briefly fasted mice; reversed light cycle, dark, for the fed mice). Units and other details as in Fig. 3. The data for the briefly fasted lymphoma-bearing mice (late tumors) are the same as those shown in Fig. 3 (x). Number of mice for the fed TB group: A, 2-3/time point, 3 terminal; B, 3 and 6 for the early and late TB groups, respectively. FFA disappearance curves were not obtained for the fed late TB mice (assumed for SAAM/CONSAM analysis, not to differ from the fed early-mid TB group; see "Experimental Protocols, Experiment 5" for additional data supporting this assumption).

noted in mice with early tumors. This may have been due to the tumor-bearing mice not having ingested as much food as controls prior to the experiment, because in later studies we have found that when lymphoma-bearing mice eat the same quantity of a single test meal as do controls, a similar decrease in plasma FFA levels occurs in both groups. Similar responses of plasma FFA concentrations to the ingestion of single test meals in EAT-bearing and control mice have been reported from our laboratory in an earlier study (13).

The fractional rate constant of plasma FFA oxidation (equivalent to the fractional catabolic rates in our model) fell in the fed as compared to briefly fasted state by 83% in control mice (from 0.96 to 0.16/min; Table 3), and by 63% in mice with early-mid tumors; however, it was only reduced by 32% (from 0.63 to 0.43/min) in mice with late tumors.

Although not relevant to the hypothesis being tested, an additional finding that might deserve further attention is our estimate of the size of the slowly mixing compartment 3 in our...
model, an undefined FA-containing compartment that exchanges FA with plasma FFA. This mass was reduced by feeding from 4500 ± 7.0% to 330 ± 30% μg-atoms FA-carbon/30 g body weight. In controls, and from 3500 ± 26% to 800 ± 44% units in early-mid stage lymphoma-bearing mice. This difference in dilution of the tracer in fed versus fasted mice probably accounts for the major differences seen in the decline of the [14C]FFA curves (fasted versus fed states) shown in Figs. 4A and 5A.

**DISCUSSION**

We have tested the hypothesis that a defect exists in the regulation of plasma FFA mobilization and oxidation in lymphoma-bearing mice. The latter were reported earlier to have a circulating peptide, LMF, which mobilized labeled triacylglycerol fatty acids from fat pads when injected into fasted-refed normal mice (1). Previous workers have explored similar hypotheses both in cancer-bearing humans (16) and mice (13). The ability of multiple glucose test meals to inhibit plasma FFA mobilization was diminished in cancer-bearing compared to control human subjects (16); however, in fasted mice refed single small test meals, plasma FFA mobilization appeared to be reduced by a single small test meal to an equal extent in previously fasted EAT-bearing and control mice (13). Oxidation of plasma FFA to CO₂, a critical measurement when estimating rates of FFA mobilization in acute studies (17), was not taken into consideration in the studies of fasted and refed EAT-bearing and control mice (13). However, in the latter study, plasma FFA levels were reduced by the test meals to the same extent in both TB and control animals. Subsequent studies of plasma FFA oxidation to CO₂ in EAT-bearing and control mice indicated that plasma FFA were not being oxidized (mobilized) at an increased rate in EAT-bearing mice (24) during the day when the animals were probably in the postabsorptive state, even though food was available *ad libitum*. Although regulation of FFA mobilization by a single test meal did not appear to be affected by tumor growth in EAT-bearing mice, the critical experiments that would evaluate rates of plasma FFA mobilization and oxidation at night when the animals eat most of their food has not been done, nor has such an experiment been done in any tumor-bearing rodent until now, to our knowledge, with the exception of respiratory quotient, nontracer studies by Lindmark *et al.* (25).

In confirmation of our earlier studies in EAT-bearing and in lymphoma-bearing mice (7, 17), we have been unable to obtain evidence of increased plasma FFA mobilization in lymphoma-bearing mice when they were studied in the briefly fasted state, even though these TB mice have been reported (1, 5) to have a potent circulating LMF. However, our present experiments, in which a reverse light cycle was used to simulate nocturnal conditions, indicate that one reason for the loss of body fat in lymphoma-bearing mice may be the continuous, largely uninhibited mobilization and oxidation of plasma FFA at night, when control mice slow down this flux by well-known, insulin-dependent regulatory mechanisms. The apparent defect in lymphoma-bearing mice, seen to a greater degree in mice with large rather than with small tumor burdens, could be due either to an inability of food to inhibit these metabolic processes or because of the markedly decreased food intake that is known to accompany lymphoma development in these mice (7).

The simplest explanation for the observed defect in the tumor-bearing mice is that they ate less food than the controls. Although such an explanation may downplay the possible roles of LMF and TNF/cachectin, it is not trivial with respect to our understanding of cancer-induced cachexia. Clearly, the continued mobilization of FFA from adipose tissue and the uninhibited oxidation of plasma FFA at night at a rate faster than in the well-nourished controls, must contribute very significantly to the loss of body fat in anorexic TB mice. Perhaps most important to investigators in this field is that these defects are not readily demonstrable in undernourished animals if the experiments are carried out in the daytime (daylight) when the animals are in the postabsorptive state. It is also noteworthy that the experimental design of Kitada *et al.* (1) that allowed them to demonstrate the action of lymphoma-derived circulating LMF centered on the use of animals that had been fasted and then refed. This suggests that LMF, as well as reduced caloric intake, may act to inhibit the regulatory effects of dietary carbohydrate in some TB mice.

Although we did not design our experiment to study the effects of tumor growth and of feeding and fasting on the kinetics of plasma-extraplasma FA exchange, we observed in our analyses of both TB and control mice that feeding caused a highly significant reduction in the size of the large slowly exchanging FA “pool” (“FA-X,” Fig. 1, compartment 3). This was evidenced by the apparent diminished disappearance of tracer from the plasma FFA compartment at early times in the fed mice. In an earlier study of briefly fasted mice (16), we suggested that the early disappearance of plasma [14C]palmitate reflects primarily a mixing process, which we show here may change significantly, depending upon the nutritional state of the animals. The present approach, based upon combined serial measurements of plasma [14C]FFA and breath 14CO₂ for short periods of time after i.v. injection of labeled FFA-albumin, is clearly able to define the oxidative component quantitatively and with high reliability, while at the same time providing useful, though less precise, estimates of parameters that relate to mixing with extraplasm FA compartments.

Further work is required with essential fatty acids and paired control mice, as well as other tumor-bearing animal strains, especially those that have been reported to lose body fat while eating normally (4, 11, 26, 27) to determine whether defective regulation of FA turnover and oxidation observed here using a nonessential FA as tracer can be generalized to essential FA turnover and metabolism, to establish whether the defect is generally found in other forms of cancer, to determine the extent to which this regulatory defect is caused by factors such as LMF and TNF/cachectin, perhaps acting as antagonists of insulin and insulin-like hormones, and to evaluate the importance of reduced caloric intake by TB mice in this phenomenon. These questions are currently under investigation.

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PLASMA FFA TURNOVER AND OXIDATION IN LYMPHOMA-BEARING MICE


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