Trafficking of Dietary Fat in Obesity Prone and Obesity Resistant Rats

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Abstract:

The trafficking of dietary fat was assessed in obesity prone (OP) and obesity resistant (OR) male and female rats. Test meals containing 1-14C palmitate were delivered through gastric feeding tubes while rats consumed a high carbohydrate diet (HCD), or after 5 days of a high fat diet (HFD). Over the subsequent 24 hours, the appearance of 14C was followed in the GI tract, skeletal muscles (SM), liver, adipose tissues (AT), and expired CO2. There was no difference in the production of 14CO2 between OP and OR rats consuming a HCD. However, after 5 days on a HFD, OR rats produced significantly more 14CO2 following the test meal than OP rats (p<0.001 females, p=0.03 males). The differential oxidation of dietary fat between OP and OR rats on a HFD was not due to differences in absorption, but rather was associated with preferential disposition of tracer to AT in OP rats. Measures of lipoprotein lipase in part explained increased tracer uptake by AT in OP rats, but were not consistent with increased SM tracer uptake in OR rats. Surprisingly, female rats oxidized more tracer than male rats irrespective of phenotype or diet. These results are consistent with the notion that differences in the partitioning of dietary fat between storage in AT and oxidation in SM and L that develop shortly after the introduction of a HFD may in part underlie the differential tendency for OR and OP rats to gain weight on this diet.

Key Words: obesity, thinness, lipoprotein lipase, gender differences, dietary fat, high fat diet.

Introduction

Obesity continues to be a major public health problem in the United States and around the world (19). Genetic and environmental factors both appear to be important in predisposing to the development of obesity. While energy intake in excess of energy expenditure is a requirement for weight gain, alterations in the disposition of dietary nutrients may also play an important role. A number of years ago, Greenwood hypothesized that excessive storage of dietary fat relative to its oxidation might predispose to fat accretion (31). She suggested that lipoprotein lipase (LPL) might play an important role in partitioning fat fuels between storage and oxidation (18), and a number of studies were then performed that measured tissue specific levels of LPL in lean, obese and reduced obese rats and humans that in general supported this idea (5, 12, 13, 15, 22, 32).

To more directly test the hypothesis that alterations in the partitioning of dietary fat are associated with obesity, labeled fat tracers have been used to follow the metabolic fate of dietary fat in vivo. Studies done in obese and reduced obese Zucker rats demonstrate less oxidation and greater adipose tissue (AT) storage of a dietary fat tracer as compared to lean Zucker rats (5, 27). Lean Sprague Dawley rats appear to preferentially traffic a dietary fat tracer to liver (L) and skeletal muscle (SM) away from AT, especially when studied in the fasted state (7). These studies support the notion that thinness is associated with greater partitioning of dietary fat towards SM and L where it may be oxidized, and that obesity is associated with greater partitioning of dietary fat towards storage in AT. Whether preferential fuel trafficking underlies the predisposition to become obese, however, requires that lipid trafficking be assessed prior to the development of obesity in a model that is more relevant to the human condition; namely one with a polygenic predisposition to become obese under environmental conditions that are similar to those that promote obesity in humans.

Such a model was developed by Levin and Keesey who used variability in weight gain
following a high fat diet (HFD) as a selective breeding tool (24, 25). This approach yielded two strains of inbred Sprague Dawley rats that have a similar phenotype when fed a high carbohydrate diet (HCD), but diverge into either susceptible (obesity prone, OP) or resistant (obesity resistant, OR) phenotypes when exposed to a HFD. This model of obesity has a number of strengths. First, it is a naturally occurring predisposition affecting both males and females. Second it has a polygenic etiology, and develops in response to a relevant nutritional factor. Third, OP rats can be reliably identified prior to the development of excessive adiposity, allowing for the examination of fuel metabolism before, and in the early process of weight gain. Finally, this model provides the opportunity to study a polygenic rodent model of thinness, potentially providing clues to adaptive mechanisms that protect against weight gain in the face of an obesigenic stimulus.

The following studies were designed to test the hypothesis that the OR phenotype is associated with trafficking of dietary fat towards oxidation in L and SM and away from storage in AT when compared to rats of the OP phenotype. To determine how genetic predisposition and diet composition affect dietary fat trafficking, OP and OR rats were studied while consuming a HCD, or following 5-days of HFD feeding. We specifically focused on this early time point in order to examine adaptive responses that arise before marked differences in body fat develop. Studies were performed in both male and female rats in an effort to see if there were gender-based differences in the adaptive responses to the HFD. Measures of LPL in SM and AT were also obtained in an attempt to explain the differences in fat trafficking. These studies reveal differences across gender as well as between OP and OR rats in the oxidation of a dietary fat tracer. Providing evidence that differences in the trafficking of dietary fat may play an important role in the predisposition, or protection from the development of obesity following introduction of a HFD.
Methods

Animals

A breeding colony of OP and OR rats was established from founder animals generously provided by Dr. Barry Levin of the Veterans Affairs Medical Center, NJ, and the New Jersey Medical School. Animals were then continuously bred and maintained at the Surgical Research Facility at Denver Health Medical Center (DHMC). A total of 80 male and female OP rats, and 80 male and female rats of the OR phenotype (total n = 160) were used for these studies; 120 for tracer studies and 40 for tissue LPL measures. Protocols were approved by the Animal Care and Use Committees at the University of Colorado at Denver and Health Sciences Center and DHMC.

Surgery and $^{14}$C palmitate meal studies

Prior to and immediately following surgery, rats had ad libitum access to a standard HCD (Lab Diet #5001: 28% protein, 12% fat, 60% carbohydrate). To facilitate the delivery of the dietary fat tracer, gastric feeding tubes were surgically implanted into the antrum of the stomach as previously described (9, 14). Following a 24-hour recovery period, and on a daily basis thereafter animals were weighed and a 3kcal liquid meal (Ensure, Ross Laboratories: 16% protein, 64% carbohydrate, 20% fat) was administered through the feeding tube to acclimate the rats to being handled and fed in this manner. Animals that lost more than 10% of their baseline weight and sustained this loss were excluded from further study.

On the third day post surgery, rats were randomly assigned to either remain on the HCD or were switched to a HFD containing 21% protein, 39% carbohydrate, and 40% fat, (33% each saturated, mono- and poly-unsaturated fats, Research Diets, Inc. #D12147). Rats were allowed to consume these diets ad libitum for 5-days. All tracer experiments were performed in the morning following a dark cycle during which rats had ad libitum access to food (fed state).
At 8:00 the morning of the study, a 3-kcal liquid meal (Ensure) supplemented with 80μl of olive oil (0.72kcal) containing 1-14C-palmitic acid (8.3 x 10⁶ dpm total dose, specific activity = 52mCi/mmol, Moravek Biochemicals) was administered to freely moving conscious rats through the previously implanted gastric feeding tubes. The test meal therefore contained 4kcal, 48% fat (65% monounsaturated, 23% polyunsaturated, and 12% saturated fat), 35% carbohydrate, and 17% protein.

After administration of the 14C labeled test meal, rats were placed in a sealed respiratory chamber. Room air was continually passed through the chamber at a flow rate of 1.0 l/min. Effluent CO₂ from the chamber was collected over 30-min intervals in 3.0-ml aliquots of a 2:1 mixture of methanol and methylbenzethonium (hyamine) hydroxide (Sigma Chemical #B2156). The 14C content of these samples was then measured with a Beckman LS6500 scintillation counter. Background activity, determined by counting a sample containing only scintillation fluid and hyamine hydroxide, was subtracted from experimental values. All expired CO₂ was collected in this manner for up to 6-hrs following tracer administration. Rats representing the 24hr time point were returned to their cages after 6-hours and a final 30 min sample of expired CO₂ was collected from animals at 24hr.

14C content in tissues

At 3, 6, and 24hrs following administration of the test meal, tissues were collected for determination of 14C content (n=5 sex/phenotype/diet/time point, total n=120). Animals were studied in a random order with respect to time and diet. At the appropriate time points, rats were removed from the respiratory chamber and anesthetized with pentobarbital (60mg/kg, ip). Skeletal muscle (SM) samples including the medial (MG) and lateral gastrocnemius (LG) and soleus (Sol) were removed, dissected free of any visible fat and connective tissue, weighed, and frozen in liquid nitrogen (entire procedure < 2min). A sample of blood was obtained from
the vena cava, and rats were then euthanized with an intracardiac injection of pentobarbital. A sample of liver (L) was removed and quickly weighed and frozen in liquid nitrogen. The remaining L was removed and weighed to determine total L weight. The gastrointestinal (GI) tract was removed, stripped completely of mesenteric fat, weighed, and placed in 0.9% saline. Perigonadal and retroperitoneal fat pads were also removed, weighed, and frozen for subsequent analyses. All samples were stored at –80°C until analyzed.

Samples of L, SM, and the entire GI tract were homogenized in ice cold 0.9% saline. Duplicate samples (0.25ml) of the homogenates (L, SM and GI) were digested with 0.5ml of tissue solubilizer overnight (Solvable, NEN) at 50°C, bleached with 50µl H2O2 and 14C content was determined by scintillation counting. Serum 14C content was determined in a similar manner. 14C content within the individual fat pads was determined after extraction of lipid with chloroform-methanol (2:1, vol/vol). Phases were separated with the addition of H2SO4 and centrifugation. The lower phase was collected and allowed to dry overnight under nitrogen, and 14C content was then measured by scintillation counting.

**LPL activity and tissue triglyceride (Tg) content**

In a subset of animals (n=5 phenotype/diet, total n=40) that reflected the previously described groups, heparin-releasable LPL activity within SM and AT were measured in fresh tissue samples as described by Nilsson-Ehle and Schotz (35). Briefly, 40-45mg of tissue was minced and incubated in a solution of phosphate buffered saline and 15µl/ml heparin for 45min at 37°C. Duplicate portions of the supernatant were removed and added to equal volumes of substrate containing 14C triolein, emulsified with lecithin and human serum as a source of Apo C2. Samples were then incubated for 45min at 37°C and the reaction was terminated by the addition of chloroform-methanol-heptane. 14C labeled free fatty acids liberated were then counted by liquid scintillation. LPL activity is expressed as nM FFA released/(g tissue min).
Triglyceride (Tg) content was determined in L and SM samples. Briefly, tissues were powdered in liquid N₂, and lipids were extracted with chloroform methanol (36). Tg content within the lipid extracts was then determined from glycerol release following acid hydrolysis (Kit 320-a Sigma, St. Louis MO).

**Calculations and statistics**

14C content of each tissue was calculated from the measured activity per gram of tissue multiplied by the total weight of the tissue. Serum 14C content was calculated as the measured 14C activity/ml of serum x 0.0385 (%body mass accounted for by serum) x body mass (10). Total body SM 14C content was calculated by multiplying the average 14C activity/g of the LG and MG by the percent SM of the entire carcass (38% of body weight) (10). Whole body AT 14C content was estimated by multiplying the averaged 14C activity/g of individual fat pads (retroperitoneal, and perigonadal) by the estimated whole body fat mass (% body fat X body weight). Percent body fat was estimated via regression equations that were determined from identical feeding experiments that examined changes in perigonadal and retroperitoneal fat pad weights relative to % body fat as determined by whole carcass analyses (data not shown). Whole body ATLPL was estimated by multiplying the average ATLPL/g X whole body fat content. All data are presented in graphical form as means ± SE. Comparisons of serum and tissue 14C content were made across phenotype (OP and OR), gender, and time (3, 6, 24hr) (Sigma Stat, Jandel Scientific, California). Differences in the cumulative production of 14CO₂ (0-3, and 0-6hr following administration of the test meal) were evaluated with a three-way ANOVA (diet, phenotype, and sex). In the event of a significant difference, multiple comparison procedures were performed with Tukey, statistical significance was accepted at p<0.05.
Results

Animal characteristics pre/post diet, surgery

Following surgery, body weights decreased in all groups (5-12g); however this decrease was transient and all animals returned to and surpassed their pre-surgical weight by the end of the experiment. At the time of test meal administration, body weights were as follows (means ± SE): OR females 203 ± 3g, OP females 235 ± 4g, OR males 294 ± 4g, and OP males 320 ± 4g. As expected, male rats were heavier than female rats of the same phenotype, and both male and female rats of the OP phenotype were slightly heavier than OR rats, males p<0.001, females p<0.001. Within each group (sex and phenotype), body weights were not significantly different in rats consuming HFD as compared to those consuming HCD.

Absorption of the fatty acid tracer

The time course of 14C disappearance from the GI tract (stomach to rectum) is depicted in Figure 1. Regardless of the phenotype or diet, female rats absorbed the tracer more rapidly than males, demonstrating lower 14C content within the GI tract at both 3 and 6 hours (p<0.001 and p=0.004, respectively). Tracer absorption was also faster in rats fed the HFD, as GI 14C content was lower in these animals at both 6 and 24 hours (p=0.032, p=0.003). A significant portion of the initial tracer load remained within the GI tract of all groups at 24 hours (~10%), and less remained in female rats than in male rats (p=0.016) (Figure 2A). While the absorption of the lipid tracer was influenced by both gender and diet composition, it did not appear to be affected by the genetic predisposition to become obese. In fact, no differences were observed between OP and OR rats at any time point.

Once absorbed from the GI tract, the lipid tracer enters the circulation in chylomicron triglycerides. Serum 14C content was highest 3hrs after test meal administration and decreased over time in all groups (see supplemental online table for complete data set). The rapid absorption of tracer in females was reflected by an elevated, albeit minor, increase in
serum $^{14}$C content that was most apparent at 3 hours as compared to males. Interestingly, the more rapid absorption of tracer in the HFD condition as compared to the HCD condition was not accompanied by an increase in the serum $^{14}$C at the 3hr time point. Rather, serum $^{14}$C content was significantly lower at 3hr in HFD fed rats ($P=0.029$), suggesting that faster absorption of the dietary fat tracer on the HFD was accompanied by more rapid clearance of the tracer by peripheral tissues. 24hr $^{14}$C serum contents are depicted in Figure 2B. A diet effect was observed within the OP rats at 24hrs, and $^{14}$C contents following HFD were significantly lower compared to rats consuming a HCD ($p<0.001, p<0.001$). In contrast, OR rats were no different or elevated in the HFD condition as compared to the HCD condition. This difference between OP and OR rats consuming a HFD at the 24hr time point was significant ($p<0.001$), and is consistent with the idea that OP rats clear the tracer more rapidly from the plasma compartment.  

$Tracer Oxidation/^{14}CO_2 Production$

Dietary fat tracer taken up by peripheral tissues could be oxidized, stored as Tg or re-secreted. The 6hr time course of $^{14}$CO$_2$ production by male and female OP and OR rats under HCD and HFD conditions is depicted in Figure 3. In all groups, $^{14}$CO$_2$ production peaked or reached a plateau by 3 hours. Regardless of diet or phenotype, females demonstrated a more rapid increase in $^{14}$CO$_2$ production and overall significantly higher levels of post-prandial oxidation of the dietary fat tracer within the first 6 hours following test meal administration. In contrast, males exhibit a slower rise in $^{14}$CO$_2$ production that was maintained relatively constant. The cumulative production of $^{14}$CO$_2$ (0-6 hours) was similar between OP and OR rats under HCD conditions (Figure 4A). In contrast, following HFD feeding the OR rats produced significantly more $^{14}$CO$_2$ than OP rats over the 6 hours following test meal administration. This was true for both female ($p<0.001$) and male rats ($p=0.03$). The difference in tracer oxidation seen in the HFD condition was largely due to divergent responses to the introduction of the
HFD in OP as compared to OR rats. Specifically, $^{14}$CO$_2$ production was reduced in OP rats consuming a HFD whereas $^{14}$CO$_2$ production was unchanged or modestly increased in OR rats in the HFD condition as compared to the HCD condition. In following, it appears that greater oxidation of the dietary fat tracer in OR rats is associated with and perhaps causally related to resistance to weight gain on a HFD.

In comparison, female rats had significantly higher levels of $^{14}$CO$_2$ production relative to males (p<0.001) irrespective of phenotype or diet condition. This sex based difference may be explained in part by more rapid absorption of the tracer from the GI tract, but likely also is a reflection of differences in the partitioning of the tracer between L, SM and AT.

*Tracer and Tg Content in L and SM*

Higher levels of tracer oxidation in female and OR rats could be the result of greater disposition of tracer to L and SM or a greater capacity for fat oxidation in these tissues. In an effort to explore these possibilities, tracer content in L and SM were measured at 3, 6 and 24hrs. Tg content in SM and L were also measured at these time points. Hepatic $^{14}$C tracer content was highest immediately after ingestion of the labeled meal and declined by 24hrs in all groups ([see supplemental online table](#)). No significant differences in hepatic $^{14}$C content were observed between OP and OR rats at any time point. In both male and female rats, exposure to the HFD resulted in a significant increase in liver Tg content when compared to Tg content in rats consuming a HCD ([Table 1](#), p<0.001). The increase in hepatic Tg content in the HFD condition was significantly greater in OP rats when compared to OR (p=0.003), and may reflect either increased storage or a reduced capacity to oxidize dietary fat in the liver of OP rats.

The other tissue that likely makes a substantial contribution to whole body dietary fat oxidation is SM. In an effort to examine the effects of fiber type on tracer disposition, SM $^{14}$C content was measured in the lateral (LG) and medial gastrocnemius (MG) muscle ([see](#))
supplemental online table) and soleus (Sol). Across all groups, the $^{14}$C content within different SM types paralleled their oxidative capacity, with the $^{14}$C content in Sol being greater than LG which was in turn was greater than MG. The time course of $^{14}$C appearance in Sol following HCD and HFD feeding is presented in Figure 5. Content of $^{14}$C in Sol was consistently and significantly higher in females as compared to males (3hr p<0.001, 6hr p<0.001, 24hr p=0.008). Contents were significantly lower at the 24 hour time point in both male (p<0.001) and female (p=0.005) rats consuming a HFD when compared to rats consuming a HCD, which could be a result of greater tracer disposition to AT in the HFD condition.

Contents of $^{14}$C within both LG and MG were used to estimate the total body SM $^{14}$C content (see supplemental online table). The total body SM $^{14}$C content at 3hr is depicted in Figure 4B. Similar to Sol, total body SM $^{14}$C content was significantly higher in females as compared to males at both 3 and 6hrs (p<0.001, p=0.008). Exposure to the HFD resulted in significantly lower total body SM $^{14}$C content in the HFD condition compared to the HCD condition at both 3 and 24hrs (p=0.019, p=0.015). The observation of a gender based difference in SM $^{14}$C content is consistent with, and may in part explain the higher $^{14}$CO$_2$ production rates observed in females. As was seen in the liver, 5 days of exposure to the HFD resulted in a significant increase in the Tg content of MG and soleus muscles in both OP and OR rats (p<0.001) (Table 1). Although not significantly different, it is interesting to note that regardless of diet, Tg contents of MG were consistently higher in both male and female OP rats. While these differences do not establish a role for skeletal muscle in the increased oxidation of the dietary fat tracer seen in female and OR rats, they are at least consistent with this hypothesis.

Tracer Content in AT

A relative reduction in the oxidation of the tracer within OP and male rats should be
associated with a reciprocal increase in the storage of dietary fat in adipose tissue (AT). To address this issue, $^{14}$C content was measured in mesenteric, retroperitoneal, and perigonadal fat pads in each group at each time point (see supplemental online table). The total $^{14}$C content within the total mesenteric pad at 3 and 24 hours is shown in **Figure 6A-B**. At both 3 and 24hr, $^{14}$C contents in the mesenteric pad were higher in the OP phenotype ($p=0.019$, $p=0.003$). The effect of the HFD was most pronounced in both male and female OP rats at the 24hr time point, and mesenteric $^{14}$C contents following HFD were increased relative to HCD ($p=0.011$, $p=0.016$).

Interestingly, the pattern of tracer accumulation within the mesenteric fat pad is very reflective of the whole body adipose $^{14}$C content estimated using $^{14}$C contents within the perigonadal and retroperitoneal fat pads. Whole body adipose $^{14}$C contents at 3 and 24hrs are depicted in **Figure 6C-D**. Indeed estimates of whole body AT $^{14}$C content demonstrated significantly more tracer in AT in the HFD condition as compared to the HCD condition at all time points (3 hr: $p=0.004$, 6hr: $p<0.001$, 24 hr: $p=0.007$). In comparison to females, male rats had significantly greater amounts of the lipid tracer directed to AT (3 hr: $p=0.019$, 6 hr: $p=0.047$, 24 hr: $p=0.005$), which is consistent with the observation of lower tracer oxidation within males. Total body AT $^{14}$C content tended to increase in all groups following HFD feeding, however, it increased to a greater extent within the OP phenotype and a phenotype within HFD effect was observed at both 6 and 24hrs ($p=0.039$, $p=0.003$). Following HFD feeding, the observation of an increased deposition of $^{14}$C tracer within the OP rats is consistent with the idea that the OP phenotype is associated with greater storage of dietary fat in AT as compared to OR rats.

**LPL Activity in SM and AT**

LPL has been viewed as the rate limiting step in the uptake of Tg derived fatty acids into SM and AT. The levels of heparin releasable LPL in AT and SM obtained from rats in this study are shown in **Table 1**. Regardless of diet, whole body ATLPL was higher in OP rats as
compared to OR. This was true for both male (p<0.001) and female rats (p=0.024). In general, the introduction of a HFD resulted in an increase in ATLPL activity, however this change was only significant in female rats (p=0.008). Interestingly, ATLPL decreased in OP males following the introduction of a HFD. In general, these results are consistent with an increased tendency to store dietary fat in OP rats both on HCD and HFD. As has been previously shown, SMLPL is more abundant in Sol as compared to MG or LG. The effects of gender and diet on SMLPL levels were inconsistent and it does not appear that changes in this enzyme in SM explain the differences seen in tracer oxidation between groups.

**Discussion.**

The results of this study support the hypothesis that the OR phenotype is associated with greater oxidation and less storage of dietary fat when compared to the OP phenotype. This difference in the handling of dietary fat did not appear to be present when rats consumed a HCD, but became apparent after the introduction of a HFD. Differences in the handling of dietary fat developed within just a few days of exposure to a HFD. While the design of these experiments does not provide definitive information on tissue uptake or turnover rates, the data are consistent with the idea that a shift of dietary fat disposition into L and SM in OR rats and into AT in OP rats underlies the observed differences in tracer oxidation. Finally, the magnitude of the difference between male and female rats was unexpected and deserves further consideration in future studies. A summary of the differences between the OP and OR phenotypes, as well as males and females are depicted in Figure 2.

A number of researchers have suggested that reduced oxidation of fat predisposes to weight gain (1, 30, 46), and a growing number of studies suggest that insulin resistance and obesity are associated with a lower skeletal muscle oxidative capacity (23, 43). One paradigm to study this issue is the weight reduced state, which is known to have a strong metabolic
propensity for weight gain. Preferential use of CHO for energy needs is thought to result in an energetically efficient deposition of excess calories, and a number of studies in both humans and rodents have shown that the weight reduced state is accompanied by a reduction in the capacity for fat oxidation (2-4, 6, 16, 28, 29, 38, 39). From this perspective, a lower capacity for fat oxidation appears to be a compensatory adaptation to weight reduction that contributes to the high incidence of relapse to the obese state. In contrast, it has been more difficult to demonstrate that a reduced capacity for fat oxidation contributes to the initial development of obesity. One of the primary challenges in addressing this issue is in the identification of subjects with a strong predisposition for weight gain prior to their actually becoming overweight or obese. To address this difficulty, one group of researchers examined non-obese relatives of those with a strong familial predisposition for obesity, reporting a reduced level of fat oxidation in these subjects (17). Another group observed that the ratio of carbohydrate to lipid being oxidized over a 24 hour period was predictive of future weight gain (46). While both of these studies demonstrate a reduction in fat oxidation in those individuals predisposed to obesity, it was not possible to determine if the limitation was in the oxidation of endogenous or dietary fat. In support of the latter possibility, Ji and Friedman found that a reduced ability to oxidize a dietary fat tracer is associated with weight gain in outbred Sprague Dawley rats (21).

Although numerous studies have examined the relationship between alterations in fat oxidation and weight gain, the current study has a number of unique features. First, to develop a more complete view of the movement of dietary fat over time, measures of tracer disposition were made in a number of relevant tissues over 24 hours following test meal administration. Second, OP and OR phenotypes were compared on a HCD and early in their exposure to a HFD to determine the relative roles of pre-existing differences and the early adaptive responses to a HFD. Finally, to our knowledge this is the first study to directly compare the dietary fat oxidation following the introduction of a HFD in both male and female rats, a variable
that appears to be quite important in determining the overall rate of dietary fat oxidation in the rat.

The studies presented were performed after a relatively brief exposure to a HFD. In contrast, numerous studies in this model and others have been done following long term exposure to a HFD, resulting in the development of an obese phenotype (25, 29). While studies using long term exposure to a HFD can provide important insights into the physiology associated with obesity, initial adaptive responses that promote or protect against weight gain may be diminished or absent by this time. To be effective in maintaining body weight, adaptive responses to a change in diet or energy imbalance likely need to occur quickly. In support of this view, recent studies by Levin have shown that leptin resistance appears to develop in OP rats within 3 days of exposure to a high energy diet (40), and Rossetti and coworkers have shown that insulin and leptin resistance develop within 3-7 days of HFD feeding (37, 45). Similarly, our observations indicate that 5-days of HFD feeding is sufficiently long enough to result in differences in the oxidation of diet derived fatty acids within OP and OR rats. Future studies might even be done in the first 1-2 days of exposure to a HFD. While the non-steady state nature of this period poses substantial experimental challenges, this may be a critical period in determining the mechanisms that underlie HFD induced weight gain.

One of the more striking findings of this study was the marked gender difference in \(^{14}\text{CO}_2\) production. Previous investigations of dietary fat trafficking in rats have not provided a direct comparison of male and female rats, and the dramatic gender differences observed in this study were surprising. Increased levels of tracer oxidation were seen in females of both the OR and OP phenotype as compared to their male counterparts. One important consideration is that, regardless of body weight, all rats were administered the same dietary tracer meal. OR rats were slightly smaller than OP rats, and males were larger than females. Correcting for total body weight or lean body mass (where tracer oxidation likely takes place) only increases the
magnitude and statistical significance of the differences seen. Greater tracer content was also observed within individual muscles and in the estimated whole body SM tracer content of female rats at all time points. In contrast, a greater fraction of the tracer label was retained in total body AT of male rats. Our current observations are consistent with our previous study using limb balance methodology in which we found that women had greater uptake of a dietary fat tracer by SM than men (20), however no differences in the post-meal generation of $^{14}\text{CO}_2$ were seen in that study. Other investigators have not found gender differences in the oxidation of a dietary fat tracer or have found greater oxidation in males (34, 41). It has been known for some time though that women have a smaller post-meal serum Tg excursion as compared to men (11) and it is possible that differential trafficking of dietary fat may in part underlie this difference. It is also possible that the rat is not a good model for studying gender differences in dietary fat metabolism as they relate to humans. However, it seems at this time that it is not entirely clear what the sex-based differences in trafficking of dietary fat are in humans, and at a minimum the important effect of gender on dietary fat oxidation identified in this study should be considered in future studies using rat models of obesity.

There are a number of important limitations to this study. The dietary fat tracer was administered intra-gastrically and $^{14}\text{CO}_2$ production was measured in breath. There are clearly a large number of steps between these two points, and it is therefore difficult to make definitive conclusions about the cause of the observed differences in $^{14}\text{CO}_2$ production. Perhaps more importantly, at the time studies were performed in the HCD condition, male rats were consuming more food than female rats. In studies conducted in the HFD condition, not only were male rats consuming more food than female rats, OP rats of both sexes were consuming more food than OR rats. In addition, tissue lipid pools were larger in OP rats when compared to OR rats at the time tracer studies were done in the HFD condition. It is not clear from the current studies what specific mechanisms are responsible for the observed differences in
tracer oxidation. It is possible that differences in food intake and tissue lipid content played an important role. Despite these limitations however, this method has been used in rats and humans by a number of investigators (26, 33, 41, 42, 44) to derive useful information about the handling of dietary fat at the whole body level. Data from studies such as this one complement studies that focus more on specific tissues. The fact that tracer recovery in CO$_2$ between groups paralleled SM content and was inversely related to AT content lends credibility to the idea that alterations in the trafficking of dietary fat underlie the observed differences in $^{14}$CO$_2$ generation. It should also be noted that these results may be specific for a palmitate tracer administered in a small test meal in the fed state, as the specific fatty acid used as a tracer and the metabolic state of the rat clearly influence the results of a meal study such as this (8). The fed state was chosen because it was hypothesized that differences between OP and OR rats would be accentuated in this condition. Another limitation is that no attempt was made to control the state of the reproductive cycle in female rats at the time of study. While this is admittedly a limitation, if anything it might be presumed to increase variability and reduce the power of the study to find meaningful differences. Since significant differences were found, it would seem to be a minor issue. In support, previous studies in humans suggest that although important, variations in the menstrual cycle do not substantially alter the trafficking of dietary fat (44).

In summary, within a few days following the introduction of a HFD, OR rats appear to oxidize more dietary fat than OP rats. OP rats in turn have a greater tendency to store dietary fat. This difference did not appear to be present prior to the introduction of the HFD. This difference in the trafficking of dietary fat was associated with maintenance of a thin phenotype in OR rats. Future studies should examine both the relative importance that changes in food intake and energy expenditure play in the development of obesity in this model and, the ways in which changes in nutrient metabolism, food intake and energy expenditure relate to each other.
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Table 1. Triglyceride content and LPL activity in peripheral tissues.

<table>
<thead>
<tr>
<th>Tissue Tg</th>
<th>Female</th>
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<th></th>
<th>Male</th>
<th></th>
<th></th>
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</thead>
<tbody>
<tr>
<td></td>
<td>OR</td>
<td>HCD</td>
<td>HFD</td>
<td>OP</td>
<td>HCD</td>
<td>HFD</td>
</tr>
<tr>
<td>Tg LG</td>
<td>3.56 ± 0.73</td>
<td>1.72 ± 0.12</td>
<td>3.11 ± 0.64</td>
<td>2.59 ± 0.20</td>
<td>1.36 ± 0.18</td>
<td>4.27 ± 1.55</td>
</tr>
<tr>
<td>MG&lt;sup&gt;a,b&lt;/sup&gt;</td>
<td>3.34 ± 0.68</td>
<td>4.72 ± 0.92</td>
<td>5.68 ± 1.08</td>
<td>7.66 ± 2.32</td>
<td>2.75 ± 0.67</td>
<td>4.52 ± 1.00</td>
</tr>
<tr>
<td>Soleus&lt;sup&gt;a&lt;/sup&gt;</td>
<td>4.69 ± 0.55</td>
<td>8.64 ± 1.84</td>
<td>6.86 ± 0.48</td>
<td>8.72 ± 1.68</td>
<td>6.17 ± 0.86</td>
<td>9.04 ± 1.61</td>
</tr>
<tr>
<td>Liver&lt;sup&gt;a,b&lt;/sup&gt;</td>
<td>3.85 ± 0.34</td>
<td>6.01 ± 0.51</td>
<td>5.08 ± 0.70</td>
<td>8.63 ± 0.66</td>
<td>4.23 ± 0.26</td>
<td>4.90 ± 0.78</td>
</tr>
<tr>
<td>Muscle LPL</td>
<td></td>
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<td></td>
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<tr>
<td>LG&lt;sup&gt;a&lt;/sup&gt;</td>
<td>2.16 ± 0.46</td>
<td>3.89 ± 1.01</td>
<td>4.78 ± 0.34</td>
<td>2.79 ± 0.80</td>
<td>3.39 ± 0.98</td>
<td>4.97 ± 0.71</td>
</tr>
<tr>
<td>MG&lt;sup&gt;a&lt;/sup&gt;</td>
<td>2.28 ± 0.66</td>
<td>4.85 ± 1.41</td>
<td>5.55 ± 0.42</td>
<td>2.60 ± 0.68</td>
<td>3.80 ± 1.81</td>
<td>4.24 ± 0.76</td>
</tr>
<tr>
<td>Soleus&lt;sup&gt;a,b&lt;/sup&gt;</td>
<td>22.5 ± 2.9</td>
<td>21.0 ± 3.3</td>
<td>24.3 ± 2.1</td>
<td>11.1 ± 2.6</td>
<td>26.6 ± 5.8</td>
<td>17.6 ± 3.3</td>
</tr>
<tr>
<td>Total body muscle&lt;sup&gt;c&lt;/sup&gt;</td>
<td>209 ± 43</td>
<td>320 ± 67</td>
<td>529 ± 35</td>
<td>287 ± 65</td>
<td>449 ± 100</td>
<td>558 ± 78</td>
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<tr>
<td>Adipose LPL</td>
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<td></td>
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<td></td>
</tr>
<tr>
<td>Perigonadal&lt;sup&gt;b,c&lt;/sup&gt;</td>
<td>11.9 ± 4.5</td>
<td>43.7 ± 6.3</td>
<td>16.7 ± 1.4</td>
<td>19.3 ± 9.7</td>
<td>15.6 ± 3.4</td>
<td>15.3 ± 5.8</td>
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<tr>
<td>Retroperitoneal&lt;sup&gt;b&lt;/sup&gt;</td>
<td>19.6 ± 5.9</td>
<td>39.8 ± 7.0</td>
<td>21.8 ± 5.9</td>
<td>20.7 ± 9.4</td>
<td>12.7 ± 3.8</td>
<td>13.9 ± 4.0</td>
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<tr>
<td>Total body adipose&lt;sup&gt;a,b&lt;/sup&gt;</td>
<td>206 ± 54</td>
<td>668 ± 92</td>
<td>580 ± 162</td>
<td>1138 ± 454</td>
<td>257 ± 64</td>
<td>354 ± 90</td>
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</table>

Triglyceride content within individual lateral gastrocnemius (LG), medial gastrocnemius (MG), soleus muscle, and liver are expressed in µmol/g wet wt. LPL Activity in three skeletal muscles and two adipose tissue depots is expressed in nmol FA g<sup>-1</sup> min<sup>-1</sup> as individual and total body estimates. Values are means ± SE, n=5/group. A significant difference was accepted at p<0.05, subscripts denote significance for a=diet, b=phenotype, c=gender. For clarity, significant interactions and the results of multiple comparison procedures are identified in the text.
Table 2. Summary of lipid trafficking in OP and OR rats.

<table>
<thead>
<tr>
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<th>Response to the HFD</th>
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<tr>
<td></td>
<td>OR</td>
</tr>
<tr>
<td>Absorption</td>
<td>↑</td>
</tr>
<tr>
<td>Clearance from Serum</td>
<td>↑</td>
</tr>
<tr>
<td>Oxidation</td>
<td>-</td>
</tr>
<tr>
<td>Trafficking to Liver</td>
<td>-</td>
</tr>
<tr>
<td>Trafficking to Muscle</td>
<td>↓</td>
</tr>
<tr>
<td>Trafficking to Adipose</td>
<td>-</td>
</tr>
</tbody>
</table>
**FIGURE LEGENDS.**

**Figure 1. Time course of \(^{14}\text{C}\) disappearance from the GI tract after meal infusion.**  
(A) Female OR and OP rats and (B) Male OR and OP rats. Absorption of an ingested lipid was assessed in obesity-resistant (OR) and obesity-prone (OP) rats that were either consuming a high carbohydrate diet (HCD, filled symbols) or after having been switch to a high fat diet for 5 days (HFD, open symbols). A meal labeled with \(^{14}\text{C}\)-palmitate was infused via a gastric feeding tube and the amount of \(^{14}\text{C}\) remaining in the GI tract (from the stomach through the colon) was assessed after 3, 6, and 24 hours. Data are expressed as means ± SE. Significant differences are denoted by like symbols within a panel or across panels. Significance was accepted at p<0.05.

**Figure 2. \(^{14}\text{C}\) content in the GI tract and serum 24 hours after meal infusion.**  
The amount of tracer in the (A) GI tract and (B) serum was assessed 24 hours after meal infusion for obesity-resistant (OR) and obesity-prone (OP) rats that were either consuming a high carbohydrate diet (HCD, filled bars) or after having been switch to a high fat diet for 5 days (HFD, open bars). Data are expressed as means ± SE for Female OR and OP rats and Male OR and OP rats.

**Figure 3. Time course of \(^{14}\text{CO}_{2}\) production within 6 hours of the meal infusion.**  
\(^{14}\text{CO}_{2}\) was collected from expired air at regular intervals within 6 hours of the meal infusion. Both Male and Female obesity-resistant (OR) and obesity-prone (OP) rats were examined after consuming (A) a high carbohydrate diet (HCD, filled symbols) or (B) after having been switch to a high fat diet for 5 days (HFD, open symbols). Data are expressed as means ± SE. No difference between the groups was observed in \(^{14}\text{CO}_{2}\) production at 24 hours (data not shown).

**Figure 4. Cumulative \(^{14}\text{CO}_{2}\) production and total skeletal muscle \(^{14}\text{C}\) content after meal infusion.**  
(A) The cumulative amount of \(^{14}\text{CO}_{2}\) collected from expired air over the 6 hour monitoring period is shown.  
(B) The amount of tracer contained in the lateral medial portions
of the gastrocnemius muscle was used in combination with regression estimates of total skeletal muscle mass (Lean body mass- Liver) in order to estimate the amount of 14C accumulated in skeletal muscle around the body at 24 hours. For both figures, Female and Male obesity-resistant (OR) and obesity-prone (OP) rats were examined after consuming a high carbohydrate diet (HCD, filled bars) or after having been switch to a high fat diet for 5 days (HFD, open bars). Data are expressed as means ± SE.

**Figure 5. Time course of Soleus 14C content after meal infusion.** (A) Female OR and OP rats and (B) Male OR and OP rats. 14C content in soleus muscles from obesity-resistant (OR) and obesity-prone (OP) rats that were either consuming a high carbohydrate diet (HCD, filled symbols) or after having been switch to a high fat diet for 5 days (HFD, open symbols). A meal labeled with 14C-palmitate was infused via a gastric feeding tube and the amount of 14C contained in the muscles was examined after 3, 6, and 24 hours. Data are expressed as means ± SE. Significant differences are denoted by like symbols within a panel or across panels. Significance was accepted at p<0.05.

**Figure 6. 14C accumulation in adipose tissue pads after meal infusion.** 14C content in adipose tissue from obesity-resistant (OR) and obesity-prone (OP) rats that were either consuming a high carbohydrate diet (HCD, filled bars) or after having been switch to a high fat diet for 5 days (HFD, open bars). 14C content in the mesenteric pad is shown at (A) 3 hours and (B) 24 hours after ingestion of the tracer meal. Mesenteric, retroperitoneal, and perigonadal tracer levels and whole body fat mass calculations were used to estimate whole body adipose tissue 14C accumulation at (C) 3 hours and (D) 24 hours after ingestion of the tracer meal. Data are expressed as means ± SE for Female OR and OP rats and Male OR and OP rats.
REFERENCES


Figure 1.
Figure 2.

A.

![Bar chart showing Total GI tract (DPM's x 10^3) for HCD and HFD groups.](chart1)

- **Female**: HCD > HFD
- **Male**: HCD > HFD

* gender p<0.001

B.

![Bar chart showing Total Body Serum 14C (DPM's x 10^3) for HCD and HFD groups.](chart2)

- **Female**: HCD > HFD
- **Male**: HCD > HFD

* phenotype p<0.001
Figure 3.
Figure 4.

A. Cumulative $^4$CO$_2$ Production (DPM x $10^3$)

- Female OR OR OP
- Male OR OP

HCD
HFD

$^*$ gender $p<0.001$
$p<0.001$
$p<0.01$

Total body muscle $^{14}$C (DPM $x 10^3$)

- Female OR OP
- Male OR OP

HCD
HFD

$^*$ gender $p<0.001$
$**$ diet $p=0.019$
Figure 5.
Figure 6.

A. Female vs. Male OR OP OR OP 3hr Mesenteric fat pad content (DPM's x10^3)

B. Female vs. Male OR OP OR OP 24hr Mesenteric fat pad content (DPM's x10^3)

C. Female vs. Male OR OP OR OP 3hr Total body adipose content (DPM's x10^3)

D. Female vs. Male OR OP OR OP 24hr Total body adipose ¹⁴C (DPM's 10^3)

* phenotype p=0.019
** gender p=0.019
*** diet p=0.004

* phenotype p=0.003
** gender p=0.003
*** diet p=0.007

p=0.011
p=0.016

p=0.003
Supplemental Table. $^{14}$C content within individual tissues.

<table>
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<tr>
<th>Tissue $^{14}$C in DPM’s</th>
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<th>6hr</th>
<th>24hr</th>
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<tr>
<td><strong>Total body serum (x's $10^3$)</strong></td>
<td>a,c</td>
<td>a</td>
<td>c</td>
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<tr>
<td>Female</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>HCD</td>
<td>59.1 ± 6.3</td>
<td>62.5 ± 7.6</td>
<td>52.7 ± 10.6</td>
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<tr>
<td>HFD</td>
<td>44.4 ± 5.4</td>
<td>42.5 ± 8.5</td>
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<td>HCD</td>
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<td>HFD</td>
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<table>
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<tr>
<th><strong>Total Liver (x's $10^4$)</strong></th>
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<tr>
<td>HCD</td>
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<td>74.7 ± 12.6</td>
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<td>HCD</td>
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<th><strong>Muscle contents (DPM/g x's $10^3$)</strong></th>
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<tr>
<td>Medial gastrocnemius</td>
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<tr>
<td>Lateral gastrocnemius</td>
<td>4.3 ± 0.6</td>
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<table>
<thead>
<tr>
<th><strong>Total body muscle (x's $10^4$)</strong></th>
<th>3hr</th>
<th>6hr</th>
<th>24hr</th>
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<td>Female</td>
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<tr>
<td>HCD</td>
<td>80.1 ± 12.2</td>
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<td>43.8 ± 7.2</td>
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<td>65.9 ± 6.6</td>
<td>40.4 ± 6.9</td>
<td>35.9 ± 7.0</td>
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<tr>
<td>Male</td>
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<tr>
<td>HCD</td>
<td>73.1 ± 16.6</td>
<td>64.4 ± 5.2</td>
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<tr>
<td>HFD</td>
<td>68.2 ± 4.5</td>
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<table>
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<th><strong>Fat pad contents (DPM/g x's $10^3$)</strong></th>
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<tr>
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<td>26.2 ± 5.6</td>
<td>61.0 ± 13.8</td>
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<td>Mesenteric</td>
<td>29.8 ± 10.6</td>
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<td>Total fat pad content (DPM x's $10^3$)</td>
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</table>

Content of $^{14}$C within individual tissues are expressed in DPM’s/g tissue. Values are means ± SE, n=5-6/group. A significant difference was accepted at p<0.05. Subscripts denote significance for a=diet, b=phenotype, c=gender.