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The role of dietary fat in obesity-induced insulin resistance

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Lackey DE, Lazaro RG, Li P, Johnson A, Hernandez-Carretero A, Weber N, Vorobyova I, Tsukomoto H, Osborn O. The role of dietary fat in obesity-induced insulin resistance. *Am J Physiol Endocrinol Metab* 311: E989–E997, 2016. First published November 1, 2016; doi:10.1152/ajpendo.00323.2016.—Consumption of excess calories results in obesity and insulin resistance and has been intensively studied in mice and humans. The objective of this study was to determine the specific contribution of dietary fat rather than total caloric intake to the development of obesity-associated insulin resistance. We used an intragastric feeding method to overfeed excess calories from a low-fat diet (and an isocalorically matched high-fat diet) through a surgically implanted gastric feeding tube to generate obesity in wild-type mice followed by hyperinsulinemic-euglycemic clamp studies to assess the development of insulin resistance. We show that overfeeding a low-fat diet results in levels of obesity similar to high-fat diet feeding in mice. However, despite a similar body weight, obese high-fat diet-fed mice are more insulin resistant than mice fed an isocaloric low-fat diet. Therefore, increased proportion of calories from dietary fat further potentiates insulin resistance in the obese state. Furthermore, crossover diet studies revealed that reduction in dietary fat composition improves glucose tolerance in obesity. In the context of the current obesity and diabetes epidemic, it is particularly important to fully understand the role of dietary macronutrients in the potentiation and amelioration of disease.

diabetes; high-fat diet; insulin resistance; insulin sensitivity; obesity

THE INCIDENCE OF OBESITY is increasing at an alarming rate, and this epidemic is driving associated comorbidities including insulin resistance and type 2 diabetes (4, 27). In mouse models, it is well established that high-fat diet (HFD) feeding induces obesity and insulin resistance (14, 35), even after a short time (20). While obesity itself plays a role in the development of insulin resistance, there are also indications that dietary fat may aggravate this process and further promote insulin resistance (6, 16, 24). However, the extent to which dietary fat specifically, rather than increased calories, contributes to obesity-induced insulin resistance is not well understood.

The mouse is a model organism widely used to study the link between obesity and insulin resistance. Ad libitum feeding mice a HFD (defined as 45–60% calories from fat) is a well-established model of obesity-induced insulin resistance. Mice eat a set mass of food per day, and, as such, a simple way to induce a hypercaloric state is to increase the caloric density of food in the diet (1, 14). Generating obesity in mice in the absence of a HFD is limited to genetic manipulation of satiety (e.g., leptin or leptin receptor deficiency) to induce hyperphagia (32). However, leptin deficiency is not representative of the majority of cases of human obesity. Pair-feeding studies [measuring the food intake in a low-fat diet (LFD) group and matching the calories given to a second group that are fed a restricted quantity of HFD] provide a starting point for dissociating the effect of calories vs. fat per se but are limited by the lack of obesity in these studies (5, 22, 23, 28, 31, 34)

In the present studies, we have used a chronic intragastric (iG) feeding model to generate obesity by overfeeding LFD. Mice are surgically implanted with an iG feeding tube and liquid diet is administered to the mice throughout the day, as previously described (7). This technique overcomes the limitations of pair-feeding studies, as mice can be overfed a hypercaloric diet in a controlled setting to allow exact matching of calories infused directly to the stomach of the mouse. This technique enables a direct comparison of the effects of dietary fat on the exacerbation of insulin resistance to isocaloric feeding of LFD in the lean and obese states.

We investigated the in vivo metabolic phenotype of lean and obese C57BL/6 male mice chronically fed a HFD and an isocaloric LFD either at a eucaloric rate to maintain leanness (LFD-regular, HFD-regular) or overfeeding (LFD-overfed, HFD-overfed) to generate obesity (Fig. 1*A*). The objective of these studies was to dissociate the effects of dietary fat vs. isocaloric feeding of LFD on the development of insulin resistance.

RESEARCH DESIGN AND METHODS

Intragastric surgery and feeding. All procedures were approved by the University of California, San Diego animal care and use committee. Male C57BL/6 mice (stock no. 000664) were purchased from Jackson at 9 wk of age. The iG model was prepared and fed by the animal core of the Southern California Research Center for ALPD and Cirrhosis (P50 AA-011999). iG catheter surgery was performed as previously described (7, 36). Briefly, mice were aseptically implanted with a long-term gastrostomy tube made of Tygon and Silastic with Dacron felt to secure the site of tube entry to the forestomach and the site of tube exit from the dorsal cervical skin. The end of the tube was connected to a 20-gauge flow-through swivel to allow dietary infusion and free movement of the mouse in a microisolator cage. Mice were housed individually and given 2 wk of postsurgical recovery before the commencement of iG feeding of liquid HFD or LFD from 12 wk of age.

Mice were infused continuously, 24 h/day, for the duration of the study with isocaloric HFD or LFD (Table 1) as previously used in this iG model (7). The percentages of energy from fat (corn oil), carbohydrate (sucrose), and protein (lactalbumin hydrolysate) used in the studies are listed in Table 1. The diets were also supplemented with vitamins and minerals (Dyets, Bethlehem, PA). Mice were fed intragastrically either at 580 $kcal \cdot kg^{-1} \cdot day^{-1}$ [= 100% for regular fed

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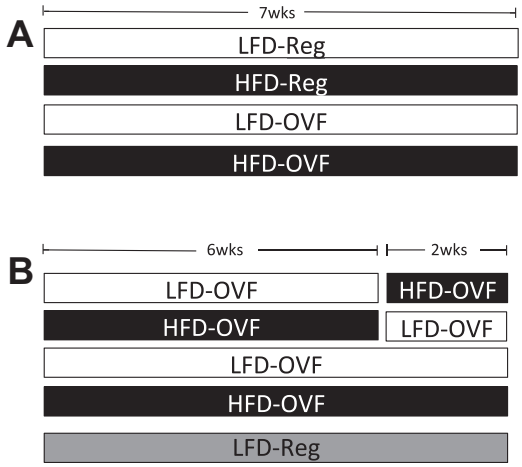


Fig. 1. Schematic diagram of intragastric (iG) feeding studies. *A*: mice were fed low-fat (LFD) or high-fat (HFD) diet via iG feeding tubes either at regular calories (Reg) to maintain lean body weight or overfed (OVF) to generate obesity. *B*: crossover diet study. Male mice were overfed LFD or HFD for 6 wk at increasing infusion rates to generate obesity (HFD-OVF and LFD-OVF). Half of each group were then switched to the opposite diet and continued overfeeding to maintain obesity for a further 2 wk (LFD-OVF→HFD-OVF or HFD-OVF→LFD-OVF).

(LFD-Reg and HFD-Reg)] or ramped gradually up to 185% above the regular rate (overfed 130% on *day 3*, 150% on *day 7*, 170% on *day 17*, and 185% on *day 28*) to generate obesity (HFD-OVF, LFD-OVF).

In vivo metabolic studies. Body weight was measured weekly to assess the development of obesity. Glucose tolerance tests were performed after 5 wk of iG feeding. Mice were fasted for 6 h before the procedure, and glucose was injected intraperitoneally (1 g/kg dextrose). Blood glucose was measured at 0, 15, 30, 60, and 90 min after injection, and blood samples were taken at 0 and 10 min to measure glucose-stimulated insulin secretion. Hyperinsulinemic-euglycemic clamps were performed in the sixth week of iG feeding, as previously described (9, 26), to quantitate the degree of systemic and tissue-specific insulin sensitivity. Briefly, dual catheters (MRE-025) were implanted in the right jugular vein, tunneled subcutaneously, and exteriorized at the back of the neck. The mice were allowed to recover for 3 days before the clamp procedure and maintained a steady body weight during this time. After a 6-h fast, blood glucose was assessed via tail nick, body weight was measured, and the mice were placed in a Lucite restrainer (Braintree Scientific, Braintree, MA). A constant infusion (5 μCi/h) of D-[3-^{3}H]glucose (Du Pont-NEN, Boston, MA) was administered. After 90 min of tracer equilibration and basal sampling at $t = -10$ and 0 min, glucose (50% dextrose, variable infusion; Abbott) and tracer (5 μCi/h) plus insulin (8 mU·kg^{-1}·min^{-1}) was infused via the jugular vein cannulas. Blood was sampled from tail clips at 10-min intervals and analyzed for glucose. Steady-state conditions (120 ± 10 mg/dl) were achieved at the end of the clamp by maintaining glucose infusion and plasma glucose concentration for a minimum of 30 min. Blood samples were taken at $t = -10$, 0 (basal), 110, and 120 (end of experiment) min to determine glucose-specific activity, insulin, and free fatty acids (FFAs). We quantified tracer-determined rates by using the Steele equation for steady-state conditions (33). Hepatic glucose production (HGP) and glucose disposal rate (GDR) were calculated in the basal state and during the steady-state portion of the clamp. At steady state, the rate of glucose disappearance, or total GDR, is equal to the sum of the rate of endogenous glucose production (HGP) plus the exogenous (cold) glucose infusion rate (GIR). The insulin-stimulated (IS)-GDR is equal to the total GDR minus the basal glucose turnover rate. After 7 wk of iG feeding mice were fasted for 6 h and killed and tissues were dissected. Group sizes were eight mice per group for four groups (LFD-Reg, HFD-Reg, LFD-OVF, HFD-OVF) (Fig. 1*A*).

Crossover diet studies. Male mice were fed LFD or HFD for 6 wk at increasing infusion rates to generate obesity. The obese overfed groups (HFD-OVF and LFD-OVF) received an increased infusion amount in a stepwise manner to match the equivalent weight gain of ad libitum HFD-fed mice (~39 g at 6 wk of HFD feeding and ~47 g by 12 wk of HFD), while the regular feeding group (LFD-Reg) received the standard caloric intake of 580 kcal·kg^{-1}·day^{-1}. Body weight was measured weekly to assess the development of obesity. After 6 wk of iG feeding each group was divided into two: one group was continuously fed the same diet, while the other group was switched to the opposite diet, while maintaining obesity, resulting in four diet groups. For example, mice overfed HFD were switched to LFD while maintaining the same number of calories (HFD-OVF→LFD-OVF). Conversely, mice overfed LFD were switched to HFD while maintaining the same number of calories (LFD-OVF→HFD-OVF). Body weight was monitored daily, and glucose tolerance tests were performed 14 days after the diet switch. Group sizes were eight mice per group for five groups (LFD-Reg, LFD-OVF, HFD-OVF, HFD-OVF→LFD-OVF, LFD-OVF→HFD-OVF) (Fig. 1*B*).

RNA isolation and quantitative RT-PCR. At death, tissues were snap frozen in liquid nitrogen and RNA was extracted as previously described (26). Total RNA was isolated with TRIzol (Invitrogen) according to the manufacturer's instructions. First-strand cDNA was synthesized with a High-Capacity cDNA Reverse Transcription Kit (Applied Biosystems). For qPCR, samples were run in a 20-μl reaction (iTaq SYBR Green supermix; Bio-Rad) with a StepOnePlus Real-Time PCR system (Applied Biosystems). Gene expression levels were calculated after normalization to the standard housekeeping gene *Actb* with the $\Delta\Delta C_t$ method (where C_t is threshold cycle) and expressed as relative mRNA levels compared with internal control. Primer sequences are shown in Table 2.

Histology. Hematoxylin and eosin staining in liver and adipose tissue sections was conducted as previously described (21). For quantification of crown-like structures (CLSs), paraffin sections were stained with antibody to F4/80 (Abcam) as previously described (40). One slide was stained per mouse and one representative image taken at ×5 magnification for each mouse ($n = 8$ mice/group). F4/80-positive CLSs were counted, and the percentage of CLSs compared with total adipocyte number was used as quantification of adipose tissue macrophage content.

Plasma measurements. Leptin, resistin, plasminogen activator inhibitor-1 (PAI-1), IL-6, monocyte chemoattractant protein-1 (MCP-1), and TNF-α plasma levels were measured with multiplex ELISA (Millipore/Linco Research). Plasma insulin levels were measured by ELISA (ALPCO). Commercially available kits were used to measure Plasma FFAs (NEFA C; Wako Chemicals) and triglycerides (TGs) (L-Type TG M, Wako Chemicals).

Statistics. All statistical analysis was performed by two-way ANOVA (with repeated measures where necessary) followed by Tukey post hoc test with GraphPad Prism version 5. P values < 0.05 were considered significant. All data are expressed as means ± SE.

Table 1. *LFD and HFD liquid diets used in iG feeding studies*

	LFD, % kcal	HFD, % kcal
Protein	25.5	25.5
Carbohydrate	65.8	34.3
Fat	8.6	40.1

The macronutrient ratios of fat (corn oil), carbohydrate (dextrose), and protein (lactalbumin hydrolysate) are shown for each diet. Vitamins and minerals (Dyets, Bethlehem, PA) were supplemented in each diet. Xanthan gum was added as a stabilizer to prevent the components of the diet from separating.

Table 2. *Sequences of primers used for gene expression qPCR*

Gene Name	Forward Primer (5′→3′)	Reverse Primer (5′→3′)
ActB	CCTGAACCCTAAGGCCAAC	GACAGCACAGCCTGGATGG
CD11b	GGTGGCAGTGTGAAGCTCT	TTGTCCCCATTCACGTCTCC
CD11c	ACACAGTGTGCTCCAGTATGA	GCCCAGGGATATGTTCACAGC
IFN-γ	TCAAGTGGCATAGATGTGGAAGAA	TGGCTCTGCAGGATTTTCATG
IL-1β	AAATACCTGTGGCCTTGGGC	CTTGGGATCCACACTCTCCAG
IL-6	CCAGAGATACAAAGAAATGATGG	ACTCCAGAAGACCAGAGGAAAT
Mcp1	AGGTCCCTGTCATGCTTCTG	GCTGCTGGTGATCCTCTTGT
Tnfa	CCAGACCCTCACACTCAGATC	CACTTGGTGGTTTGCTACGAC
Arginase	ATGGAAGAGACCTTCAGCTAC	GCTGTCTTCCCAAGAGTTGGG
ACC	CTGACGTATACTGAACTGGTGTTGGATG	TTTCCAGGCTACCATGCCAATCTC
FAS	CCAGACAGAGAAGAGCCATGGAGG	CCAATGAGGTTGGCCCAGAACTCC
Scd1	TTCTTGCGATACACTCTGGTGC	CGGGATTGAATGTTCTTGTCGT
Srebp1c	GCCGTGGTGAGAAGCGCACAGCCC	CAAGACAGCAGATTTATTCAGCTTTGC

RESULTS

Isocaloric iG feeding of LFD and HFD at the regular infusion rate (580 kcal·kg^{-1}·day^{-1}) to maintain leanness resulted in similar body weights between these two groups of mice. The increased infusion rate (up to 185% over the regular infusion rate, 1,073 kcal·kg^{-1}·day^{-1}) resulted in the development of obesity in both the HFD and LFD iG-fed mice (Fig. 2*A*). There was no significant difference in body weight between the obese LFD-OVF and HFD-OVF mice.

After 5 wk of iG overfeeding, HFD-fed mice developed impaired glucose tolerance (Fig. 2*B*) and insulinemia (Fig. 2*C*) compared with isocaloric LFD-fed mice. Remarkably, obese LFD-OVF mice showed levels of glucose tolerance comparable to lean LFD mice despite differences in body weight (~14 g, 30%) between these groups.

To determine the effects of dietary fat on both systemic and tissue-specific insulin sensitivity, we conducted hyperinsulinemic clamp studies (Fig. 3). The body weights at the time of the clamp (6 wk) are shown in Fig. 3*A*. As expected, obesity resulted in impaired systemic insulin sensitivity compared with the lean dietary control (LFD-OVF vs. LFD-Reg and HFD-OVF vs. HFD-Reg) (Fig. 3*B*). Interestingly, in the obese state, high dietary fat induced significantly impaired insulin sensitivity compared with isocaloric LFD-OVF mice. Furthermore, HFD-Reg-fed lean mice displayed systemic insulin sensitivity comparable to LFD-OVF obese mice, and these lean groups were both very insulin sensitive as shown by the high GIR values (Fig. 3*B*) and near-maximal % HGP suppression (Fig. 3, *C–E*). The impaired insulin sensitivity induced by HFD in the obese state may be attributable to decreased suppression of HGP in the liver (Fig. 3, *C–E*). Decreased insulin-stimulated glucose disposal rate (IS-GDR), a measure of in vivo glucose uptake, was also observed in the HFD-OVF mice relative to LFD-OVF mice, but this was not statistically significant (Fig. 3, *F* and *G*). Finally, no significant differences were observed in suppression of FFAs during the clamp, suggesting that adipose tissue insulin sensitivity is not affected by feeding HFD compared with isocaloric LFD (Fig. 3, *H* and *I*).

At the end of the 7-wk feeding study, both obese OVF groups had greater epididymal adipose [epididymal white adipose tissue (eWAT)] mass than lean Reg-fed mice (Fig. 4*A*). The eWAT weight was comparable between the lean LFD-Reg and HFD-Reg groups, while the HFD-OVF group had significantly increased eWAT mass (+0.45 g) compared with the LFD-OVF group.

Gene expression analysis of eWAT revealed that obese mice fed HFD had significantly increased inflammatory gene expression compared with LFD-OVF obese mice (Fig. 4*B*). Mcp-1 was significantly increased in eWAT from obese HFD-OVF compared with obese LFD-OVF mice. Increased inflammation (interferon γ) was also observed in subcutaneous adipose tissue (Fig. 4*C*). The extent of macrophage infiltration into adipose tissue was measured by staining for macrophage marker F4/80 in adipose tissue sections (Fig. 4*D*). Quantification of the

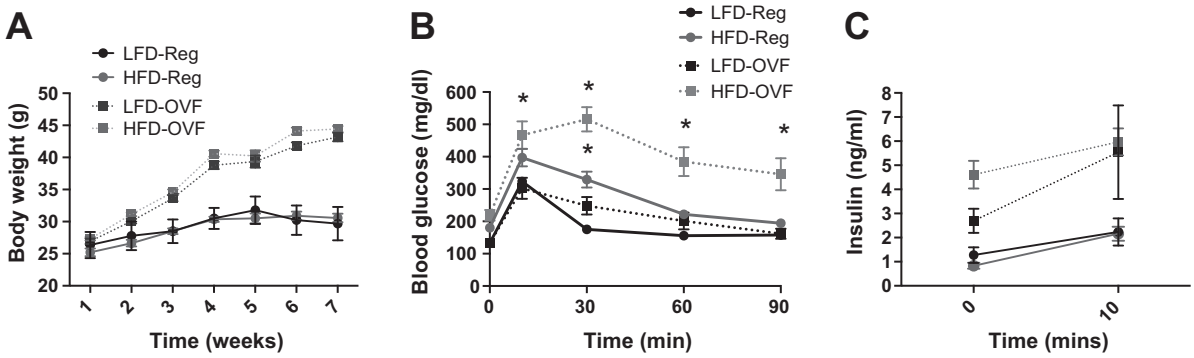


Fig. 2. Body and tissue weights of intragastric (iG)-fed mice. *A*: weekly body weights. *B*: glucose tolerance test in mice after 5 wk of iG. *C*: plasma insulin concentration before glucose injection (0) and 10 min after injection. Mice were fed low-fat (LFD) or high-fat (HFD) diet via iG feeding tubes either at regular calories (Reg) to maintain lean body weight or overfed (OVF) to generate obesity. Mice were injected with glucose (ip) at a dose of 1 mg/kg, and plasma glucose was measured at the times indicated. Data are average ± SE of 8 mice/group. Repeated-measures 2-way ANOVA with Tukey post hoc test, $P < 0.05$.

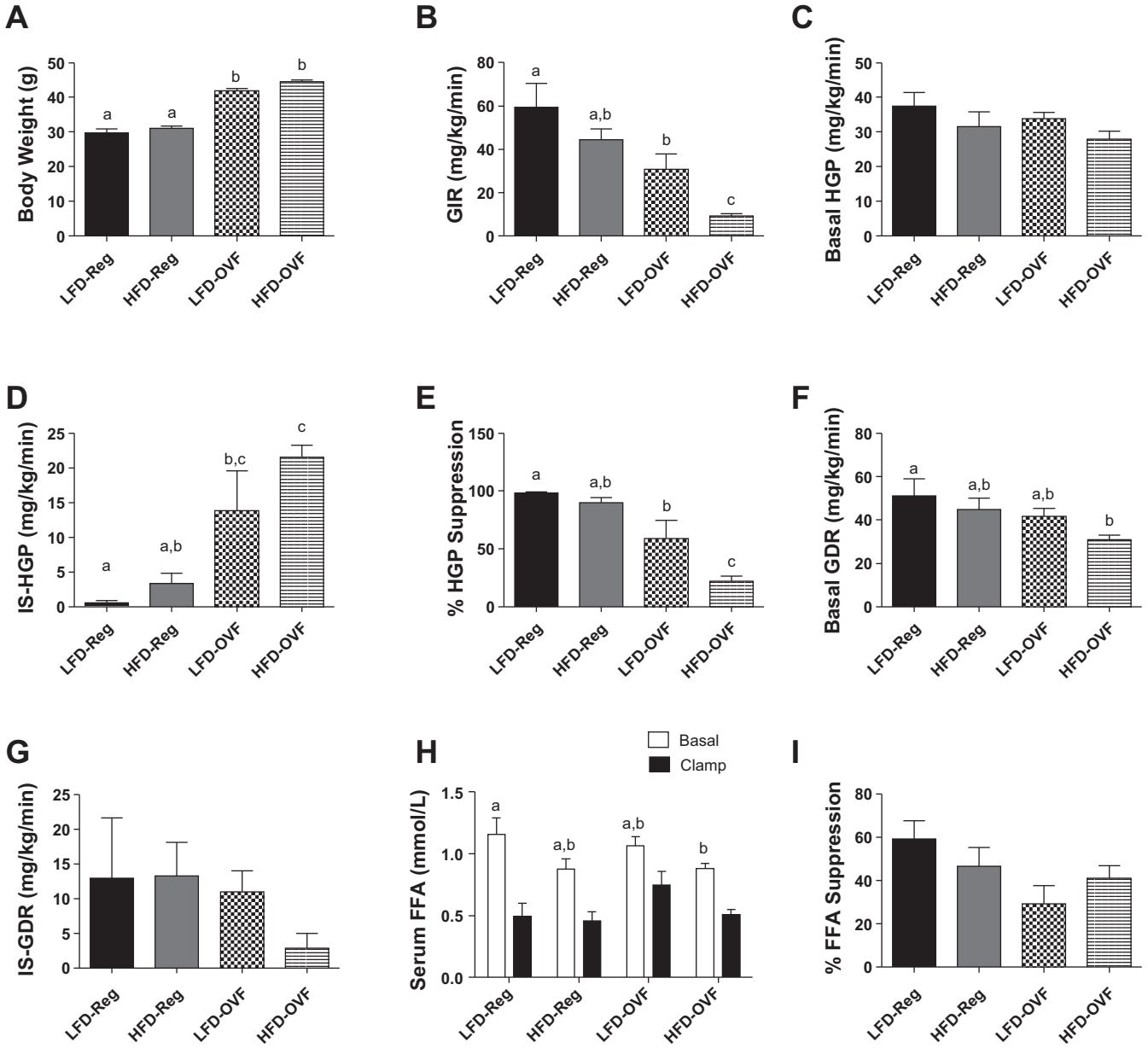
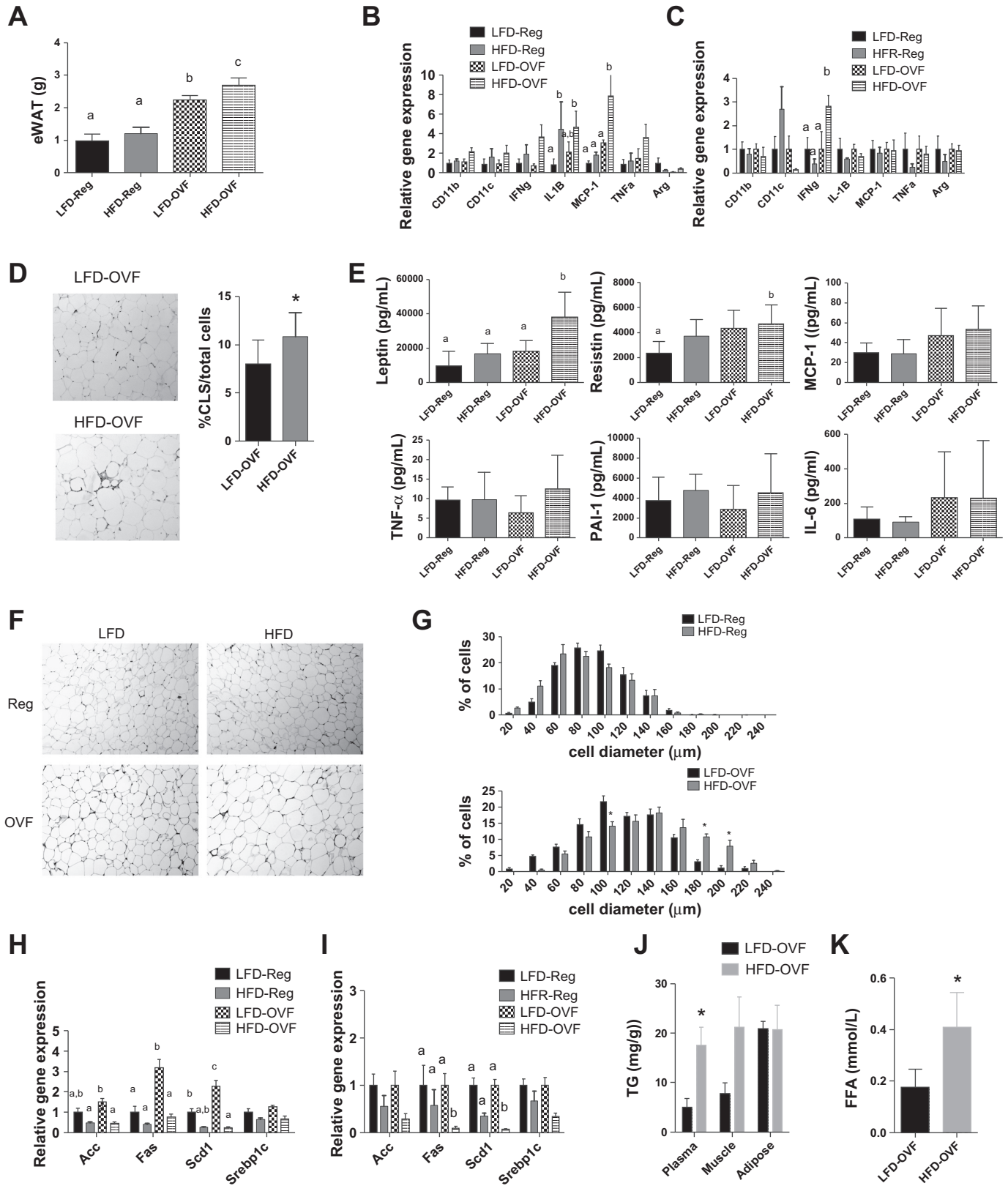


Fig. 3. Hyperinsulinemic-euglycemic clamps in obese mice isocalorically fed high-fat (HFD) or low-fat (LFD) diet. *A*: body weight. *B*: glucose infusion rate (GIR). *C*: basal hepatic glucose production (HGP). *D*: insulin-stimulated HGP (IS-HGP). *E*: % hepatic glucose (HGP) suppression. *F*: basal glucose disposal rate (GDR). *G*: insulin-stimulated glucose disposal (IS-GDR). *H*: basal and clamp serum free fatty acid (FFA) levels. *I*: % FFA suppression. Hyperinsulinemic-euglycemic clamps were performed on mice that were fed LFD or HFD diet via intragastric (iG) feeding tubes either at regular calories (Reg) to maintain lean body weight or overfed (OVF) to generate obesity. Data are average ± SE of 8 mice/group. Two-way ANOVA with Tukey post hoc test, $P < 0.05$. Values labeled with different letters are significantly different.

number of CLSs revealed higher numbers of macrophages in the HFD-OVF adipose tissue compared with the LFD-OVF adipose tissue (Fig. 4*D*). Circulating inflammatory makers were also measured, and HFD-OVF mice had significant increased leptin levels compared with LFD-OVF mice. Other markers of inflammation (TNF-α, PAI-1) were slightly higher in the HFD-OVF group compared with LFD-OVF mice but did not reach statistical significance (Fig. 4*E*).

Excess calories consumed get stored as fat, and hematoxylin and eosin staining of the adipocytes revealed that overfeeding increased adipocyte cell size. Representative images show that HFD-OVF drives a further increase in adipocyte cell size compared with LFD-OVF mice (Fig. 4, *F* and *G*). At the transcriptional level, quantification of gene expression involved in lipogenesis revealed increased expression of genes that drive adipogenesis including acetyl-CoA carboxylase (ACC), fatty acid synthase (FAS), and stearoyl-CoA desaturase-1 (SCD-1) and suggests increased de novo synthesis of fatty acids in adipose tissue in both eWAT (Fig. 4*H*) and subcutaneous adipose depots (Fig. 4*I*). Plasma levels of TG and FFA were also significantly increased in the HFD-OVF group compared with LFD-OVF (Fig. 4, *J* and *K*).

At the end of the 7-wk feeding study both obese OVF groups had greater liver mass than lean Reg-fed mice (Fig. 5*A*).



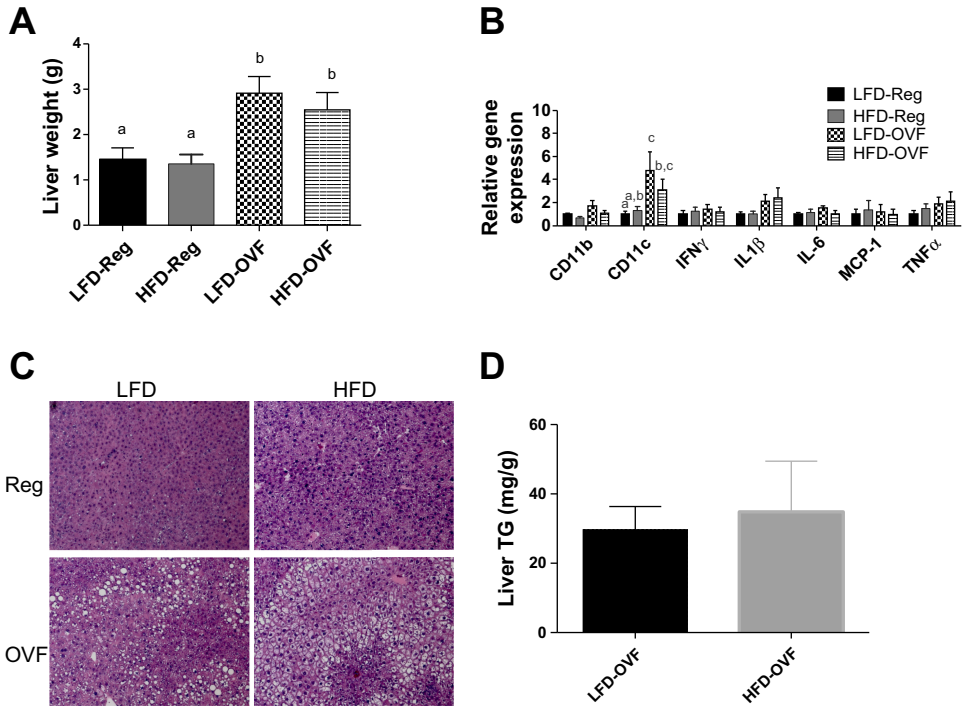


Fig. 5. Analysis of liver from mice fed low-fat (LFD) or high-fat (HFD) diet via intragastric (iG) feeding tubes either at regular calories (Reg) to maintain lean body weight or overfed (OVF) to generate obesity. *A*: liver weight. *B*: inflammatory gene expression. *C*: hematoxylin and eosin staining of the liver. *D*: liver triglyceride (TG) levels. After 7 wk of iG feeding mice were euthanized and tissues weighed. Real-time qPCR was used to determine relative gene expression between groups. Data are average ± SE of 8 mice/group. Repeated-measures 2-way ANOVA with Tukey post hoc test, $P < 0.05$. Values labeled with different letters are significantly different.

However, liver weight was not different between the lean LFD-Reg and HFD-Reg groups or the LFD-OVF and HFD-OVF groups. Inflammatory gene expression was increased in obese groups compared with lean controls, but there was no difference between LFD-OVF and HFD-OVF mice (Fig. 5*B*). Hematoxylin and eosin staining of the liver revealed increased hepatocyte ballooning in the HFD-OVF-treated group compared with LFD-OVF control-treated mice (Fig. 5*C*), but no significant differences in TG levels were observed (Fig. 5*D*).

To determine whether reduction of dietary fat, while maintaining obesity, can acutely improve the insulin-resistant state, we performed crossover diet studies (Fig. 6). Obese mice chronically overfed with HFD or isocalorically overfed LFD for 6 wk were then switched to the opposite diet (but maintaining obesity; Fig. 6*A*). Two weeks after the diet switch from LFD to HFD, overfed mice had further impaired glucose tolerance compared with continuously overfed LFD mice (Fig. 6, *B* and *C*). Furthermore, switching diet from HFD to LFD improved glucose tolerance relative to continuously overfed HFD mice (Fig. 6, *B* and *C*). Epididymal adipose weight (Fig. 6*D*) and liver weight (Fig. 6*E*) were increased in obese groups relative to the lean (LFD-Reg) control but were not significantly different between obese overfed diet groups.

DISCUSSION

In this study we examined the contribution of HFD compared with isocaloric LFD to the development of obesity and insulin resistance. We found that overfeeding of isocaloric LFD and HFD result in similar levels of obesity. However, despite a similar body weight, obese HFD-fed mice are more insulin resistant and hyperinsulinemic than mice fed an isocaloric LFD. Hyperinsulinemic-euglycemic clamp studies confirmed that HFD overfeeding further potentiated the insulin-resistant state compared with obese LFD-OVF mice. At the tissue level, HFD overfeeding resulted in significant impairment of insulin-stimulated suppression of HGP compared with LFD-OVF mice. In addition, a trend in reduction in insulin sensitivity of muscle and adipose tissue in HFD-OVF mice compared with LFD-OVF mice was observed by reduced GDR and reduced suppression of FFA production, respectively. In addition, histological analysis of the liver showed more severe hepatocyte ballooning in the HFD-OVF liver sections compared with LFD-OVF mice. We noted an increase in epididymal adipose tissue mass, an increase in adipocyte cell size, and increased inflammation and macrophage infiltration in HFD-OVF mice compared with LFD-OVF mice. Therefore, in-

Fig. 4. Epididymal adipose tissue after 7 wk of isocaloric feeding HFD or LFD. *A*: epididymal white adipose tissue (eWAT) weight. *B*: inflammatory gene expression in subcutaneous adipose tissue. *C*: inflammatory gene expression in epididymal adipose tissue. *D*, *left*: representative immunohistochemistry images of eWAT sections stained with anti-F4/80 Ab. *Right*: no. of crownlike structures (CLSs) was calculated as % of total cells in each section. *E*: systemic inflammatory protein levels. *F*: hematoxylin and eosin staining of adipose tissue. *G*: average adipocyte cell diameter in all groups. *H*: adipogenesis gene expression in epididymal adipose tissue. *I*: adipogenesis gene expression in subcutaneous adipose tissue. *J*: triglyceride (TG) levels in plasma, muscle, and epididymal adipose tissue. *K*: plasma free fatty acid (FFA) levels. Mice were fed LFD or HFD via intragastric (iG) feeding tubes either at regular calories (Reg) to maintain lean body weight or overfed (OVF) to generate obesity. After 7 wk mice were euthanized and tissues weighed. Real-time qPCR was used to determine relative gene expression between groups. ACC, acetyl-CoA carboxylase; FAS, fatty acid synthase; SCD-1, stearoyl-CoA desaturase-1; Srebp1c, sterol regulatory element-binding protein 1c. Data are average ± SE of 8 mice/group. Two-way ANOVA with Tukey post hoc test, $P < 0.05$. Values labeled with different letters are significantly different. *Denotes significance where only 2 groups (LFD-OVF and HFD-OVF) are compared.

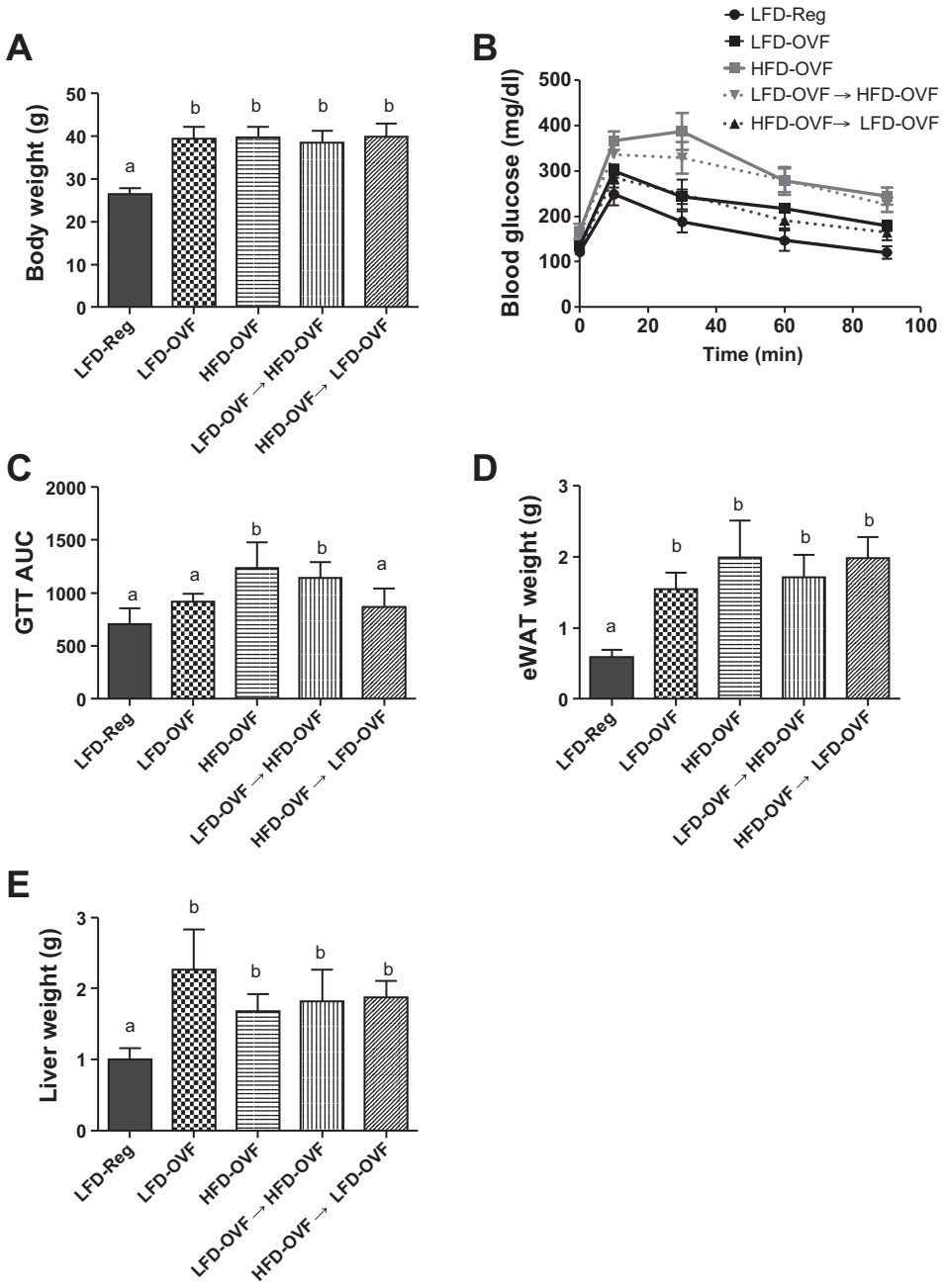


Fig. 6. Crossover diet study. Male mice were overfed LFD or HFD for 6 wk at increasing infusion rates to generate obesity (HFD-OVF and LFD-OVF). Half of each group were then switched to the opposite diet and continued overfeeding to maintain obesity for a further 2 wk (LFD-OVF→HFD-OVF, or HFD-OVF→LFD-OVF). *A*: body weight. *B*: glucose tolerance test (GTT) 2 wk after diet switch. *C*: area under the curve (AUC) of GTT. *D*: eWAT weight. *E*: liver weight. Data are average ± SE of 8 mice/group. Repeated-measures 2-way ANOVA with Tukey post hoc test, $P < 0.05$. Values labeled with different letters are significantly different.

creased proportion of calories from dietary fat further potentiates insulin resistance in the obese state.

It is well established that HFD feeding in mice induces obesity and impairs insulin sensitivity (13, 14, 20, 35). The HFD-induced insulin resistance occurs rapidly (within 3 days) when mice are placed on HFD (20). When the diet is then switched from HFD to LFD, mice lose weight and thus regain insulin sensitivity (12, 21). The improvement in insulin sensitivity is seen in all major insulin target tissues, including muscle, liver, and adipose tissue, after the weight loss induced by diet switch (21). In these studies it has been difficult to determine the specific effect of LFD feeding on the improvement of insulin sensitivity, as the weight loss is a confounding factor.

In these crossover diet studies we are able to change the diet from HFD to LFD while maintaining obesity. After a chronic feeding paradigm of iG infusion for 6 wk, switching the diet from HFD-OVF to LFD-OVF resulted in a significant improvement in insulin sensitivity within 2 wk, without weight loss. Furthermore, the opposite dietary switch from LFD-OVF to HFD-OVF potentiated the level of insulin resistance in these equally obese mice. Therefore, these studies revealed that in obese mice chronically fed HFD the insulin-resistant state was readily reversible by reducing the dietary fat content in the absence of weight loss. Interestingly, in human studies diet modification with very moderate weight loss (5–10%) is sufficient to dramatically improve insulin sensitivity (29, 38). The fact that slight weight reduction through dietary modification,

as opposed to achieving ideal body weight, can restore insulin sensitivity suggests that there is a disconnect between degree of obesity and insulin sensitivity (8).

To our knowledge, this is the first study that combines the use of the iG overfeeding model with hyperinsulinemic-euglycemic clamp studies to study the direct effects of dietary macronutrients on insulin sensitivity in the obese state in wild-type mice. While previous studies have acutely infused lipid intravenously over the course of hours and determined the effect on insulin resistance (10, 15, 17, 18, 37), we chronically infuse a HFD intragastrically over the course of weeks, which may have different effects due to the route and duration of administration.

HFD feeding results in higher circulating levels of fatty acids that activate TLR4 signaling and induce downstream inflammatory signaling and insulin resistance (30). Therefore, it is likely that in our HFD-OVF model the impaired insulin sensitivity in the HFD-OVF mice is a result of increased activation of the TLR4 pathway, inducing the increased inflammation in adipose tissue and liver and driving the development of insulin resistance. Furthermore, it is known that HFD feeding promotes intestinal absorption of lipopolysaccharides (LPS) from the gut microflora, which also plays an important role in the development of insulin resistance (3, 11), and thus future studies will be needed to address the impact of HFD-OVF on intestinal permeability and the microbiome.

In previous studies pair feeding was often used to match caloric consumption of HFD to ad libitum LFD, but these studies are limited by the fact that all the mice are lean (5). To induce overfeeding of LFD (or normal chow) in mice, genetic manipulation of satiety hormone receptors can be used, but these results are always confounded by dysregulation of hormonal homeostasis (39). The iG model overcomes these limitations and provides a controlled feeding system to study the effect of dietary macronutrients in obese mice. However, there are some important differences between this iG feeding system and normal feeding behavior. The iG infusion is a continuous chronic infusion, 24 h/day, and thus does not elicit the usual meal patterns and associated hormonal signaling that drives feeding behavior. Furthermore, the liquid corn oil diet is very different from the standard HFD fed to mice and the macronutrient profile of the average human diet (25). Corn oil has a very high ratio of ω-6 to ω-3 fatty acids, which drive potent proinflammatory effects and the development of insulin resistance (2, 19). Future studies will be needed to address the effects of saturated and unsaturated dietary fats and dissect the role of the ω-6-to-ω-3 ratio in the development of insulin resistance in this obese context. This highly controlled iG feeding system could also be used to address many other important questions in the field of circadian rhythms where feeding can be administered at any time throughout the day/night.

In the context of the current obesity and diabetes epidemic, it is particularly important to fully understand the role of dietary macronutrients in the potentiation and amelioration of disease.

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DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the author(s).

AUTHOR CONTRIBUTIONS

D.E.L., R.G.L., H.T., and O.O. conceived and designed research; D.E.L., R.G.L., P.L., A.J., A.H.-C., N.W., I.V., and O.O. performed experiments; D.E.L., N.W., and O.O. analyzed data; D.E.L. and O.O. interpreted results of experiments; D.E.L. and O.O. prepared figures; D.E.L. and O.O. drafted manuscript; D.E.L. and O.O. edited and revised manuscript; D.E.L., H.T., and O.O. approved final version of manuscript.

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