A Low-Fat Diet Has a Higher Potential Than Energy Restriction to Improve High-Fat Diet-Induced Insulin Resistance in Mice

Martin Muurling, Miek C. Jong, Ronald P. Mensink, Gerard Hornstra, Vivian E.H. Dahlmans, Hanno Pijl, Peter J. Voshol, and Louis M. Havekes

Previous studies have shown that energy restriction (ER) or low-fat (LF) diets have beneficial effects on high-fat (HF) diet-induced obesity and non–insulin-dependent diabetes. However, comparison between ER and low-fat diet regarding the effect on insulin resistance and lipid metabolism has not been reported. After inducing insulin resistance by HF feeding for 20 weeks, male C57BL/6J mice were divided into 3 groups. For a period of 12 weeks, group 1 received energy restriction (70% of ad libitum, HF diet), group 2 LF diet, and group 3 maintained on HF diet. Body weight and energy intake were reduced equally in ER and LF feeding. Plasma insulin levels were decreased on LF feeding, but were unchanged on ER, when compared with HF feeding. Glucose tolerance and insulin sensitivity tests revealed that insulin sensitivity was improved more efficiently by LF feeding than on ER. Plasma triglyceride (TG) levels were lower on LF feeding compared with ER and HF feeding. Measurement of hepatic very–low-density lipoprotein (VLDL)-TG production revealed a lower production after LF diet feeding or ER compared with HF diet feeding. In summary, our data show that LF diet has a higher potential than ER to improve HF diet-induced insulin resistance, and that there is an association between improvement of insulin resistance and decrease of TG levels.

THE HIGH PREVALENCE of overweight, around 55% to 63% (body mass index $\geq$ 25 kg/m^2) in the United States population,[1,2] continues to be a public health concern throughout Western societies. Overweight and, in particular, obesity is a major risk factor for the development of type 2 diabetes mellitus and associated pathologic states, such as hypertension, dyslipidemia, and atherosclerosis.[3] Numerous studies have shown that consumption of a high-fat (HF) diet directly relates to the development of obesity. In humans, it has been demonstrated that dietary fat intake positively correlates with body fat content, independent of total energy intake.[4-6] Furthermore, it was reported that increases in the proportion of fat in the diet predicted subsequent increases in body mass.[7] HF feeding is also found to be associated with type 2 diabetes mellitus, both dependent,[8] and independent[9] of obesity.

Weight loss, induced by either energy restriction (ER) or reduction of dietary fat, has been shown to improve glycemic control and lipid metabolism in obese individuals.

When put on ER, obese individuals lose weight and show lowering of plasma glucose, insulin, and very–low-density lipoprotein (VLDL)-cholesterol.[10,11] When saturated fatty acids are substituted with mono- or polyunsaturated fatty acids, additional effects were observed. The lipoprotein profile of the obese individuals changes to less atherogenic, with lower LDL-to-high-density lipoprotein (HDL) ratio.[10] Diets with low fat (LF) content were also able to reduce weight of obese individuals even consumed ad libitum.[12-15] Further, it has been shown that reduction of dietary fat was able to improve insulin sensitivity in humans.[16-18]

A relationship between HF diet and obesity and type 2 diabetes mellitus has also been found in rodents. One animal model that is particularly susceptible to dietary effects is the C57BL/6J mouse. This animal will develop obesity and type 2 diabetes mellitus when fed a HF diet and closely resembles common forms of the human disease after developing obesity.[19-21]

Comparable to human studies, in rodents, beneficial effects on body weight, glycemic control, and type 2 diabetes have been found upon ER and reduction of dietary fat. Several studies performed in rodents show that HF diet-induced obesity and insulin resistance can be improved by reducing dietary fat content.[22-24] In rats, it has been shown that reducing dietary fat from 40% to 30% of total energy rapidly reverses insulin sensitivity, determined by glucose tolerance.[23] Another study performed in rats shows an almost linear relationship between the amount of dietary fat and glucose tolerance, with a threshold between 30% and 40% energy provided by fat.[24] Limited studies on ER in rodents show that decreased amounts of previous ad libitum fed HF diets result in decreased body weight, but effects on insulin resistance and glycemic control are not reported.[25,26]

Experimental evidence that dietary fat favors the development of obesity and its related disorders in human is more convincing than epidemiologic studies.[27] The limitation of those experimental studies in man is the relative short duration of the dietary intervention. Since obesity is caused by many factors, such as energy intake, dietary fat, energy expenditure, gender, genetic, social, and cultural factors, it is very difficult to design a fully controlled long-term study in humans.[27-29] In contrast to humans, mice are very useful in that respect; one can easily standardize energy intake, diet, gender, and also genetic background in mice.

Although ER and LF diets are able to reverse obesity, the

From the TNO-Prevention and Health, Gaubius Laboratory, Leiden; Department of Human Biology/NUTRIM, Maastricht University, Maastricht; and the Departments of Cardiology and Internal Medicine, Leiden University Medical Center, Leiden, The Netherlands.

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Address reprints requests to Louis M. Havekes, PhD, TNO-Prevention and Health, Gaubius Laboratory, Zernikedreef 9, 2333 CK Leiden, The Netherlands.

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effectiveness of ER versus LF feeding on the improvement of insulin sensitivity and plasma lipids in obesity has never been directly compared in one study design. Therefore, the present study was undertaken to directly compare the ability of both ER and LF feeding to improve insulin resistance and plasma lipids in HF diet-induced obese mice. For these purposes, C57BL/6J mice were fed a HF diet for a period of 20 weeks to induce obesity and insulin resistance. Thereafter, mice were subjected to a dietary intervention for 12 weeks, receiving either ER (30%), a LF diet, or maintained on a HF diet. Our results show that independent of energy intake and weight reduction, LF feeding improves insulin sensitivity and lowers plasma lipids more effectively than ER. Furthermore, the reduction in plasma triglyceride (TG) levels upon LF feeding is the result of a significant decrease in hepatic VLDL production and probably more efficient clearance of VLDL particles.

MATERIALS AND METHODS

Study Design

Male C57BL/6J mice (n = 40) were housed in a temperature-controlled room on a 12-hour light/dark cycle and had free access to water and a standard mouse chow diet. At the age of 12 weeks, they were fed a HF diet, in which 45.3% of the energy is derived from fat (Table 1) for a period of 20 weeks, followed by a diet intervention period of 12 weeks. Mice were divided into 3 groups according to the diet intervention they received. The first group was maintained on the HF diet and functioned as the control group (HF). Mice in the second group were housed individually and put on ER, thereby receiving 70% of their average ad libitum food intake of the HF diet. The food intake was calculated for every individual mouse by multiplying the average food intake by 0.7. The third group was given a LF diet ad libitum (Table 1). All diets were manufactured by Hope Farms, Woerden, The Netherlands.

During the study, body weight and energy intake were measured weekly. To estimate energy intake, food intake was assessed by weighing the food in each cage dispenser, including the food that was spilled on the floor of the cage. Then, energy intake was calculated by multiplying the amount of food intake by the energy content of the diet.

After 20 weeks of HF feeding and at the end of the diet intervention period, plasma levels of glucose, insulin, free fatty acids (FFA), TG, and cholesterol were measured to determine the effects of the diet intervention on those parameters. In addition, at the end of the diet intervention period, experiments were performed to determine the effects of the dietary interventions on tolerance to a glucose bolus, insulin sensitivity, and hepatic VLDL-TG production. Unless stated otherwise, all experiments took place at 1:00 PM with food withdrawn at 9:00 AM.

Table 1. Composition of the Diets

Variable	HF Diet	LF Diet
Energy content (kJ/g)	20.0	15.9
Macronutrients		
As % of energy		
Fat	45.3	17.0
CHO	30.1	58.9
Proteins	18.3	24.0
As % of weight		
Fat	24.1	7.2
CHO	35.1	54.6
Proteins	21.4	22.3

Plasma Glucose, Insulin, Lipid, and Lipoprotein Analysis

To measure plasma glucose, FFA, TG, cholesterol, and insulin, blood (200 μL) was obtained from the tip of the tail at 12 weeks of age (t = 0), at the start (t = 20), and at the end of the diet intervention period (t = 32) after 4 hours fasting. The blood was collected in paraoxinized capillary tubes[30] and kept on ice. The samples were then spun (13,000 rpm) at 4°C for 3 minutes, and the separated plasma was immediately assayed for the respective parameters. Plasma glucose was determined by a commercially available kit (#315-500; Sigma Diagnostics, Diesenhofen, Germany). Fasting insulin was measured by using a radioimmunoassay kit (Sensitive Rat Insulin Assay; Linco Research, St Charles, MO). Levels of total cholesterol and TG, corrected for free glycerol, were determined using commercially available enzymatic kits (Boehringer Mannheim GmbH #2336691, Mannheim, Germany, and Sigma GPO-Trinder kit 337-B, St Louis, MO). FFA was measured enzymaticaly with a NEFA-C kit (Wako Chemicals GmbH, Neuss, Germany).

For fast protein liquid chromatography (FPLC) fraction of lipoproteins, 50 μL pooled plasma of the respective groups was injected onto a Superose 6 column (3.2 $\times$ 30 mm, Smart-System, Amersham Pharmacia, Uppsala, Sweden), and eluted at a constant flow rate of 50 μL/min with phosphate-buffered saline (PBS) (pH 7.4, containing 1 mmol/L EDTA). Fractions of 50 μL were collected and assayed for total cholesterol as described above.

Glucose Tolerance and Insulin Sensitivity Test

At the end of the diet intervention period, the animals were subjected to a glucose tolerance and insulin sensitivity test. For the glucose tolerance test, mice were fasted for 4 hours. Baseline blood samples (30 μL) were then collected as mentioned above (t = 0). Subsequently, the mice received an intraperitoneal injected bolus (2 g/kg) of 10% (wt/vol) D-glucose solution, and additional blood samples (30 μL) were drawn at 15, 30, 60, and 120 minutes after injection. Plasma glucose levels were measured at the different time points as described above. To examine glucose tolerance, the area under the glucose tolerance-curve was calculated.

The insulin sensitivity test was performed similarly as the glucose tolerance test, but hereby 1.0 U insulin per kilogram mouse was administered intraperitonealy as a 0.2 U/mL solution. The insulin sensitivity was determined by the responsiveness of plasma glucose during the insulin sensitivity test.

Hepatic VLDL-TG Production

Hepatic VLDL-TG production was estimated in mice that were fasted for 4 hours. Subsequently mice were anaesthetized (0.5 mL/kg Hypnorm; Janssen Pharmaceutica, Berchem, Belgium and 12.5 mg/g midazolam; Roche B.V., Mijdrecht, The Netherlands) and received an intravenous injection of 500 mg/kg Triton WR 1339 (Sigma) using a 15% (wt/wt) solution in 0.9% NaCl. Previous studies have shown that plasma VLDL-TG clearance is virtually completely inhibited under these circumstances.[31] Blood samples were drawn at 0, 30, 60, 90, 120, and 180 minutes after Triton injection, and plasma TG levels were measured at the different time points as described above. Plasma TG concentrations were related to body weight, and hepatic VLDL-TG production was calculated from the slope of the curve and expressed as μmol/kg/h.

Data Analysis

Statistical analysis was performed using analysis of variance (ANOVA) and post hoc Bonferonni *t* procedure. Statistical significance was set at $P < .05$.

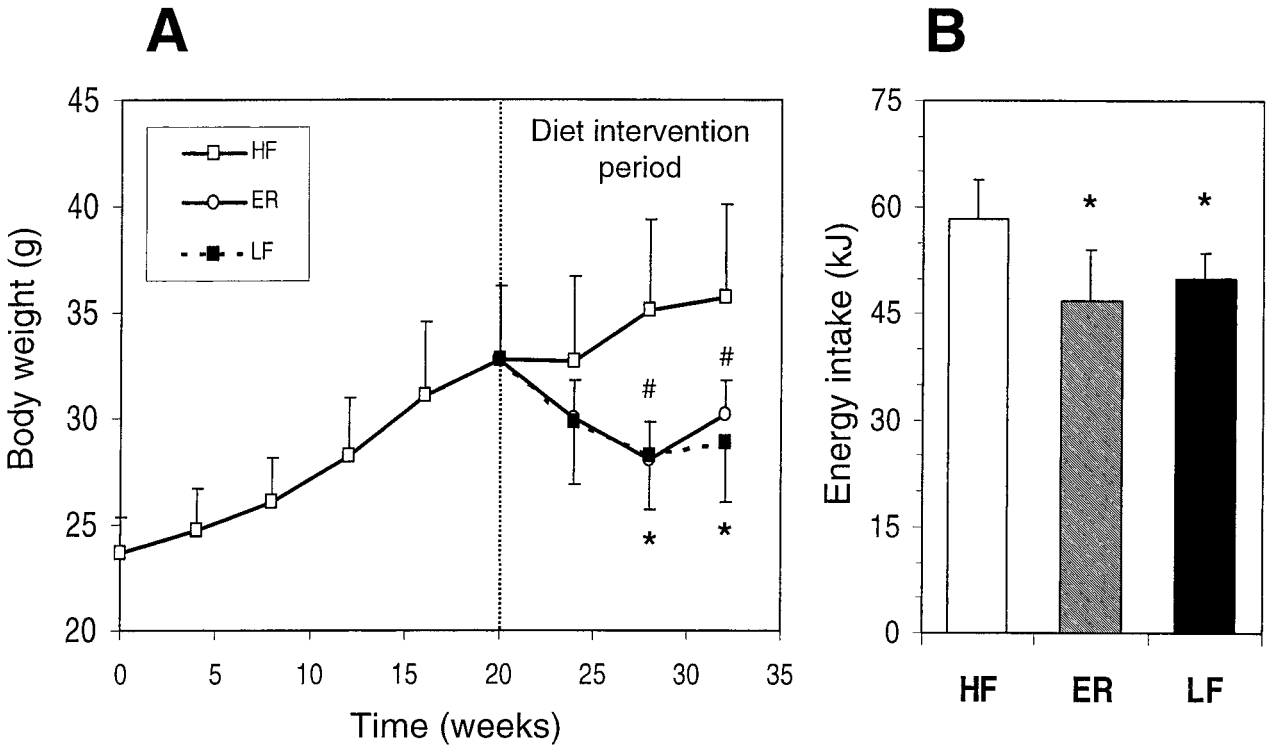

Fig 1. (A) Growth curves of wild-type mice during HF feeding and diet intervention. Mice were fed a HF diet for 20 weeks, followed by a diet intervention period of 12 weeks, during which they received 30% ER, a LF diet, or were maintained on a HF diet. Values represent the mean ± SD. Number of mice: HF (until t = 20), n = 47 mice; HF (t = 20 to 32), n = 18; ER, n = 7; and LF, n = 15. *$P < .05$ HF *v* ER, #$P < .05$ HF *v* LF, using 1-way ANOVA and post hoc Bonferonni *t* procedure. (B) Average daily energy intake during the diet intervention period. Energy intake was determined as described in Materials and Methods section. (□) HF; (▒) ER; and (■) LF. *$P < .05$ ER/LF *v* HF, using 1-way ANOVA and post hoc Bonferonni *t* procedure.

RESULTS

Effects of Diet Intervention on Insulin Sensitivity

Figure 1 shows that body weight increases upon HF diet feeding during the experimental period, while body weight of mice receiving either ER or the LF diet significantly decreased during the diet intervention period (Fig 1A). The marked reduction in body weight upon ER and LF diet feeding is probably caused by the significant reduction in the average daily energy intake as shown in Fig 1B.

To determine whether energy restriction and LF diet feeding were associated with changes in insulin sensitivity, plasma glucose and insulin levels were measured in the respective groups after 4 hours fasting. After 20 weeks of HF feeding, the mice had become hyperinsulinemic, according to the increased plasma insulin levels when compared with the start of the HF feeding (data not shown). However, plasma glucose levels remained unchanged after 20 weeks of HF feeding (data not shown). Twelve weeks of ER and LF diet feeding caused a significant decrease in plasma glucose levels compared with prolonged HF diet feeding (32 weeks) (Table 2). Surprisingly, LF diet feeding resulted in a marked decrease in the plasma insulin level, whereas no changes occurred upon ER compared with HF diet feeding.

To investigate whether the changes in plasma glucose levels upon ER and LF diet feeding were associated with an improved glucose clearance and insulin response, glucose tolerance and insulin sensitivity tests were performed in the respective groups. As shown in Fig 2A and B, mice that

Table 2. Comparison of Plasma Glucose, Insulin, FFA, TG, and Total Cholesterol After Diet Intervention

	No.	Glucose (mmol/L)	Insulin (ng/ml)	FFA (mmol/L)	TG (mmol/L)	TC (mmol/L)
HF	18	11.4 ± 0.9	2.3 ± 0.4	0.46 ± 0.08	0.20 ± 0.05	4.3 ± 0.5
ER	7	7.3 ± 1.6*	2.0 ± 0.8	0.38 ± 0.05	0.30 ± 0.11	3.0 ± 0.5*
LF	15	9.3 ± 1.5*	1.6 ± 0.2*	0.49 ± 0.10	0.14 ± 0.03*	2.9 ± 0.5*

NOTE. Male wild-type mice were maintained on a chow diet. At the age of 12 weeks, mice received a HF diet for a period of 20 weeks, followed by a diet intervention of 12 weeks receiving 30% ER or a LF diet, or were maintained on HF feeding. At the end (t = 32) of the diet intervention period, plasma glucose, insulin, FFA, TG and TC were measured after 4 hours fasting, as described in Materials and Methods.

*$P < .05$ ER/LF *v* HF, using 1-way ANOVA and post hoc Bonferonni *t* procedure.

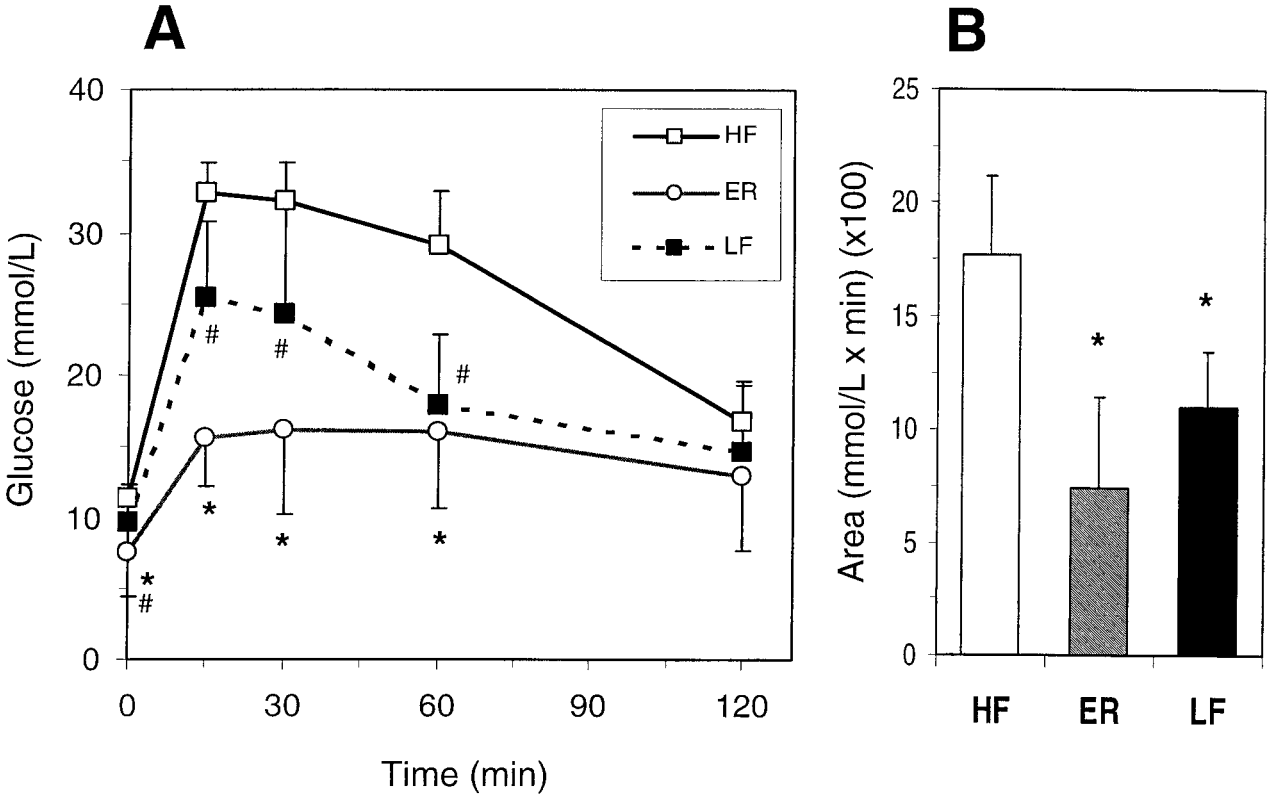

Fig 2. Glucose tolerance test in wild-type mice after the diet intervention. (A) Mice were fasted for 4 hours and then injected intraperitoneally by glucose (2 g/kg as a 10% D-glucose solution). Changes in plasma glucose were monitored in time. Values represent the mean ± SD. Number of mice: HF, n = 10; ER, n = 7; LF, n = 8. *$P < .05$ HF *v* ER and #$P < .05$ HF *v* LF, using 1-way ANOVA and post hoc Bonferonni correction. (B) Area under the curve during the glucose tolerance test. *$P < .05$ ER/LF *v* HF, using 1-way ANOVA and post hoc Bonferonni *t* procedure.

received the ER or LF diets were much more efficient in clearing a bolus of glucose than the HF diet-fed mice. Half an hour after injection of insulin, plasma glucose levels were significantly lower in both groups of the diet intervention as compared with the HF diet-fed group (Fig 3A). Remarkably, however, plasma glucose levels were decreased to such a low level in the LF diet-fed mice that these mice had to be rescued from severe hypoglycemia by an intraperitoneal bolus of glucose. Mice from the LF group that suffered from hypoglycemia were withdrawn from the experiment; 3 mice between t = 30 and 60 minutes, the remaining between t = 60 and 120 minutes (Fig 3B).

Effects of Diet Intervention on Plasma Lipids

To determine whether the improvement in insulin sensitivity upon ER and LF diet feeding was associated with changes in plasma lipids, plasma FFA, TG, and cholesterol levels were measured in the respective groups (Table 2).

During the diet intervention period, no changes in plasma FFA occurred in all 3 groups. After the diet intervention, plasma TG levels were significantly decreased in the LF group, but not in the ER group compared with the HF diet group (Table 2).

Plasma cholesterol levels significantly decreased upon dietary intervention with ER and LF diet feeding (Table 2). This decrease in plasma cholesterol upon dietary intervention is reflected by a reduction of cholesterol in the intermediate-density lipoprotein (IDL)/LDL fraction in both the ER and LF groups (Fig 4).

To investigate the mechanism underlying the reduction in plasma lipid levels and the decrease in IDL/LDL fraction upon ER and LF diet feeding, we measured hepatic VLDL-TG production by intravenous injection of Triton WR-1339 (Fig 5). Mice on ER and LF diet feeding exhibited a significantly lower VLDL-TG production rate compared with the insulin-resistant HF diet fed mice (74.4 ± 9.1 and 85.5 ± 23.5 *v* 124.7 ± 21.5 μmol TG/kg/h, respectively).

DISCUSSION

We aimed at studying the effects of ER and LF diet on HF diet-induced insulin resistance and accompanying changes in plasma lipids levels. Our results reveal that weight loss by either ER or LF feeding results in improvement of insulin resistance, but that the LF diet has a greater effect in this respect. Compared with HF feeding, ER had no effect on plasma insulin levels, but glucose levels were decreased, whereas LF diet feeding resulted in a decrease of both plasma insulin and glucose levels. This suggests that insulin resistance is more diminished by LF feeding than by ER. More reason for this suggestion is yielded by the glucose

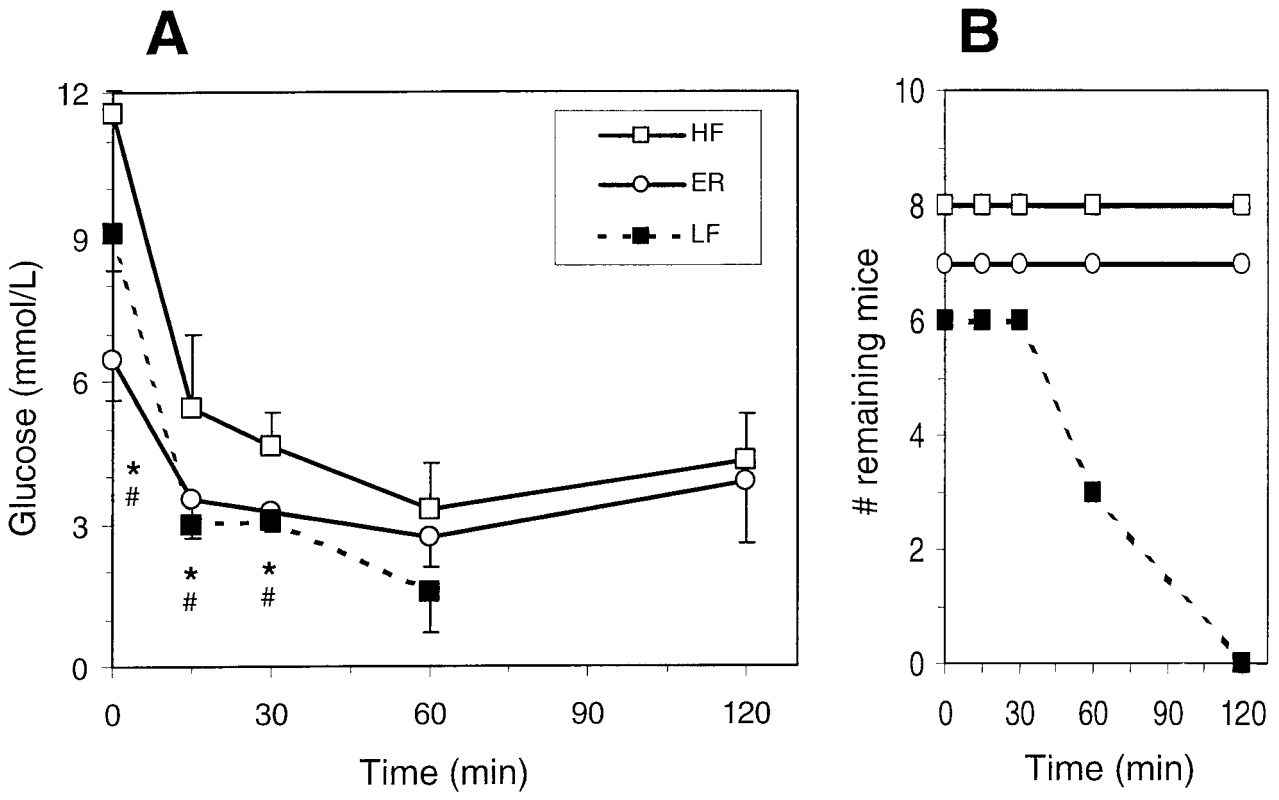

Fig 3. Insulin sensitivity test in wild-type mice after the diet intervention. (A) Mice were fasted for 4 hours and injected intraperitoneally by insulin (1.0 U/kg as a 0.2 U/mL solution). Changes in plasma glucose were monitored in time as described in Materials and Methods. Values represent the mean ± SD. Number of mice: HF, n = 8; ER, n = 7; LF, n = 6. *$P < .05$ HF *v* ER and #$P < .05$ HF *v* LF, using 1-way ANOVA and post hoc Bonferonni *t* procedure. During the experiment, mice on LF diet showed severe hypoglycemia and were withdrawn from the experiment before t = 120 minutes. Therefore, no data was obtained at t = 120 minutes from the mice on LF diet. (B) "Drop-out curve" during the insulin sensitivity test. Mice that suffered from hypoglycemia were withdrawn from the experiment after being rescued by a bolus of glucose.

tolerance and insulin sensitivity tests. During the glucose tolerance test, both the LF and ER groups showed, compared with the HF group, improved tolerance to the administered bolus of glucose, but there are no significant differences between the 2 groups. Upon administration of insulin, the LF mice became hypoglycemic and had to be rescued by a bolus of glucose. In contrast to the LF mice, the HF and ER mice did not show this phenomenon. This observation indicates that LF mice are more sensitive to the action of insulin than HF and ER and adds to our conclusion that LF feeding leads to higher insulin sensitivity than ER.

The observation described above is also observed in another mouse model, namely, the hyperlipidemic transgenic mouse expressing the human Apolipoprotein E*3Leiden (data not shown). This model exhibits a human-like lipoprotein profile, ie, VLDL- and LDL-cholesterol, with higher cholesterol IDL/LDL.[32]

The only difference between ER and LF groups is the amount of energy derived from fat and carbohydrates (CHO). Both diets were isocaloric, but the ratio fat:CHO was 1.5 and 0.29 in ER and LF, respectively, whereas the fat source (corn oil), was the same for both groups. ER received 70% of the HF diet in which 46% of the energy is provided

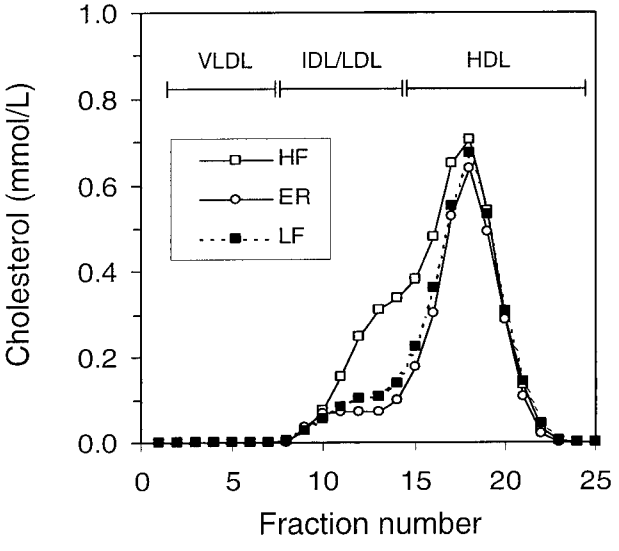

Fig 4. Lipoprotein profiles. Plasma of wild-type mice was pooled (50 μL) and separated based on size by FPLC. Total cholesterol of each individual fraction was measured as described in Materials and Methods.

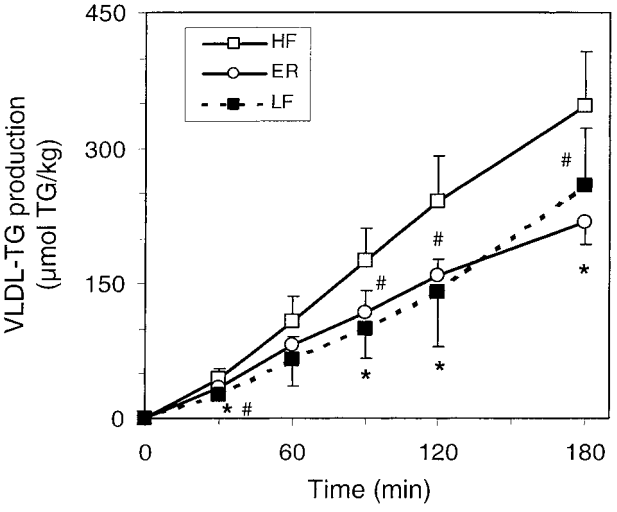

Fig 5. Hepatic VLDL-TG production in wild-type mice after diet intervention. Mice were fasted for 4 hours, anesthesized, and injected with Triton WR-1339 (500 mg/kg). Changes in plasma TG were monitored in time as described in Materials and Methods. Values represent the mean ± SD. Number of mice: HF, n = 8; ER, n = 5; LF, n = 5. *$P < .05$ ER *v* HF and #$P < .05$ LF *v* HF, using 1-way ANOVA and post hoc Bonferonni *t* procedure.

by corn oil, while this is only 14.7% for the LF diet. So, it seems quite obvious that the higher potential to improve insulin resistance by the LF diet is associated with the lower fat content of the diet, independent of body weight, energy intake, and fatty acid composition of the diet.

To determine the effects of improved insulin resistance on plasma lipids, we measured plasma FFA, TG, and cholesterol levels after the diet intervention period. Plasma TG levels decreased after LF diet feeding compared with HF diet feeding, while no changes occurred after ER. The changes in plasma TG cannot be explained by a decrease in plasma FFA, since no differences were observed between the 3 different groups. In the LF diet-fed animals, the decrease in plasma TG was accompanied with decreased plasma insulin, suggesting a link between improvement of insulin resistance and decrease in plasma TG level. A noticeable observation was that on both ER and LF diet feeding the hepatic VLDL-TG production was reduced when compared with HF diet feeding. This can be explained by a lower flux of FFA from adipose tissue to the liver due to a reduction in the amount of adipose tissue, since body weight is reduced in both ER and LF groups. We observed no significant change in hepatic TG content (data not shown) between the different dietary groups, excluding this as a possible explanation for the low VLDL-TG production found in the ER and LF group.

Decreased peripheral FFA flux seems to protect to diet-induced insulin resistance in several transgenic mouse models, eg, human apolipoprotein C1 overexpression[33] and VLVDL-receptor knock-out.[34] We also reduced peripheral FFA flux by decreasing dietary fat intake (by ER and LF feeding). Although the total amount of energy consumed by ER and LF groups was similar, total fat intake (0.55 ± 0.07 g and 0.19 ± 0.01 g/d, respectively) and energy derived from fat (20.7 ± 2.5 kJ and 7.2 ± 0.3 kJ/d, respectively) was much lower in the LF animals. Because of the lower fat content, the FFA flux is more reduced by LF than ER. Thus, the improvement of insulin resistance observed in LF could be explained by the higher reduction of peripheral FFA flux.

The observed difference between ER and LF on plasma TG levels seems to be related to more efficient clearance of the VLDL particles, since hepatic VLDL-TG production is similar in both groups. Thus, improved clearance might be due to the improved insulin sensitivity observed in the LF group.

That type 2 diabetes is associated with disturbed TG metabolism and is changed by reversing the insulin resistant state by, eg, abolishing obesity, is clear from several studies in mice and humans.[35-37] In mice, obesity associated with type 2 diabetes can lead to elevated plasma levels of TG by increased production and secretion of VLDL-TG by the liver. This effect is secondary to an increase in de novo lipogenesis and increased plasma FFA delivery to the liver, in combination with a decreased FFA oxidation.[35] Acute hyperinsulinemia, by administration of insulin, inhibits the hepatic VLDL-TG production by decreasing the FFA flux to the liver.[36] However, chronically insulin-resistant hyperinsulinemic obese individuals are resistant to the suppressive effect of insulin on VLDL apolipoprotein B production.[36] In addition, insulin resistance can lead to decreased lipoprotein lipase activity in skeletal muscle, an effect that partly explains the lipoprotein abnormalities observed in obese and diabetic patients.[37] Thus, hyperlipidemia observed in type 2 diabetes with or without obesity can be explained by a higher production of TG on the one hand and a decreased clearance (lipolysis) on the other hand.

Our data seem to be in contrast with clinical studies in humans, which suggests that LF high-CHO diets increase plasma TG.[38-40] However, those studies were under isocaloric- and weight-maintaining conditions, in contrast with our study.

The findings observed in our study are generally in line with other studies performed in mice and rats.[22-26] Both ER and LF diets led to improvement of glycemic control, but the LF diet had a stronger positive effect on insulin sensitivity. Since the C57BL/6J mice resemble common features of the human disease after developing obesity[19-21] and the effects due to the dietary interventions are roughly in line with human studies,[10-18] it might be suggestive to extrapolate the findings in our study to the human situation, although we are aware of the pitfalls.[27]

In conclusion, we found in mice that a LF diet has a higher potential than ER to improve HF diet-induced insulin resistance. Additionally, LF diet feeding improves plasma lipid levels, which seems a concomitant effect of the improvement of insulin resistance, ie, decreasing hepatic VLDL-TG production and probably increasing clearance (lipolysis).

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