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Differential effect of weight loss with low-fat diet or high-fat diet restriction on inflammation in the liver and adipose tissue of mice with diet-induced obesity

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Abstract

Objective—We studied the effects of weight loss induced by either a low-fat normal diet (ND) or restriction of high-fat diet (HFD) on hepatic steatosis, inflammation in the liver and adipose tissue (AT), and blood monocytes of obese mice.

Methods—In mice with HFD-induced obesity, weight loss was achieved by switching from HFD to ND and maintaining on ND ad libitum or by restricting HFD intake to match body weight of mice with ND-induced weight loss. After diet interventions for 4 weeks, hepatic steatosis, hepatic and AT inflammation, and blood CD11c⁺ monocytes were examined.

Results—At 4 weeks after switching diets, body weight was reduced by 23% from baseline. To achieve the same reduced body weight required restricting calorie intake from HFD. Weight loss with either ND or HFD restriction decreased body fat mass and ameliorated liver steatosis; both effects were greater with ND-induced weight loss than HFD restriction-induced weight loss. Weight loss with ND but not HFD restriction normalized blood CD11c⁺ monocytes and attenuated hepatic inflammation assessed by chemokine and CD11c expression. In contrast, weight loss with

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HFD restriction significantly reduced chemokine levels and CD11c⁺ cells in AT compared to obese controls, and tended to reduce AT chemokines and CD11c⁺ cells more than ND-induced weight loss.

Conclusion—In mice with diet-induced obesity, weight loss with ND was superior in alleviating hepatic inflammation and steatosis, whereas weight loss with HFD calorie restriction provided greater amelioration of AT inflammation.

Keywords

diet; inflammation; obesity; weight loss

Introduction

Obesity increases the risk for cardiovascular disease (CVD) [1], type 2 diabetes, and other diseases such as cancer. However, the underlying mechanisms remain largely unknown. Chronic inflammation evidenced by accumulation and activation of macrophages/dendritic cells (DCs) and T cells occurs in adipose tissue (AT) and the liver in obesity and may contribute to the development of obesity-related diabetes and CVD [2–8]. Obesity-associated hepatic inflammation may also contribute to the increased risk for hepatocellular carcinoma in obesity [9]. Therefore, attenuation of inflammation associated with obesity is expected to exert beneficial effects on obesity-linked diseases and thus be a potential novel therapeutic strategy for these diseases.

Diet-induced weight loss has been widely used to treat obesity [10,11]. However, the optimal diet for reducing body weight and improving insulin sensitivity is still debated. The amount of dietary fat and fat composition of the diet (saturated fatty acids [FAs] versus polyunsaturated FAs [PUFAs], especially n-3 PUFAs such as docosahexaenoic acid [DHA] and eicosapentaenoic acid [EPA]) seem to be important [12–15], but previous data about the effects of various weight loss diets on weight change and metabolic parameters were inconsistent [14,15] or indicated that different compositions of fat, protein, and carbohydrates in weight loss diets did not impact insulin sensitivity [15]. It remains unknown whether various weight loss diets have differential effects on inflammation, which may be involved in development of CVD and diabetes, and on hepatic steatosis, which may contribute to obesity-linked insulin resistance and to the risk for liver cirrhosis and even hepatocellular carcinoma [9].

In addition, although most previous studies compared the effects of various weight loss diets by matching caloric intake [15], it is clinically more practical to monitor body weight than to monitor dietary caloric intake of patients. Therefore, in the present study, we examined the effects of the same degree of weight loss obtained with different diets on inflammation in the liver and AT, hepatic steatosis, and blood CD11c⁺ monocytes, which are increased in obesity and hypercholesterolemia and contribute to atherogenesis [3,16], in mice with high-fat diet (HFD)–induced obesity. Weight loss was induced either by a low-fat normal diet (ND) fed ad libitum or by restriction of HFD consumption; the diets differed not only in total fat content but also in fat composition, including saturated FAs and unsaturated FAs [2].

Materials and Methods

An expanded methods section is provided in supplementary material.

Animals and diets

Obesity was induced in male C57BL/6J mice (Jackson Laboratory, Bar Harbor, ME) by feeding HFD (Dyets 112734; Dyets Inc., Bethlehem, PA) for 24 weeks; mice on ND (Picolab Rodent Diet 5010; Purina Mills, St. Louis, MO) throughout the study were used as lean controls (NDC). In HFD, 42.0% of kcal was from fat; in ND, 12.7% of kcal was from fat. HFD contained about 10 times more saturated FAs than ND but lacked DHA and EPA; ND contained DHA and EPA [2].

After HFD for 24 weeks, obese mice were divided into 3 treatment groups for 4 weeks of comparative diet interventions: (1) an obese control group that was maintained on HFD ad libitum (HFC); (2) a weight loss group that was switched from HFD to ND and maintained on ND ad libitum (WL-ND); and (3) a weight loss group that received restricted amounts of HFD to maintain the same body weight as WL-ND on the previous day (WL-HFDr). Body weight and food intake were recorded daily during the 4-week comparative diet interventions. Whole body composition was examined 2 days before the end of interventions by using a PIXI-mus Small Animal Densitometer (LUNAR, Madison, WI). After 4 weeks of comparative diet interventions, mice were sacrificed under anesthesia, and perigonadal fat pads, liver, and fasting plasma were collected. To make the fasting period comparable among the 4 groups of mice before sacrifice, HFD was provided to WL-HFDr mice at 2:00 pm, and food was removed from WL-ND, NDC, and HFC mice when WL-HFDr mice finished eating the HFD (around 6:00 pm). After fasting overnight, the mice (from all the groups) were sacrificed, and the above tissues and blood were collected early the next morning. All animal studies were approved by the Institutional Animal Care and Use Committee of Baylor College of Medicine.

Liver histology and measurement of hepatic triglyceride content

Liver tissue was fixed in Z-fix (Anatech Ltd, Battle Creek, MI), embedded in paraffin, and sectioned. The sections were stained with hematoxylin and eosin using standard protocol. Hepatic triglyceride (TG) content was measured in liver lipid extract with a Triglyceride Test Kit (Wako Chemicals USA, Inc. Richmond, VA).

Quantification of mRNA and protein

RNAse protection assay (RPA) or quantitative reverse transcription polymerase chain reaction (qRT-PCR) was performed to examine mRNA levels of target molecules in total RNA isolated from AT and liver [2,3]. Protein levels of MCP-1 and adiponectin were examined in mouse plasma by ELISA.

Flow cytometric analysis

Collagenase digestion was performed to fractionate mouse AT into adipocytes and stromal/vascular cells (S/Vs). Flow cytometric analysis was conducted to quantify T cells and macrophages/DCs in AT and CD11c⁺ monocytes in blood [2,3].

Metabolic measurements

Fasting plasma levels of glucose, insulin, cholesterol, TGs, and total free fatty acids (FFAs) were measured and the homeostasis model assessment of insulin resistance (HOMA-IR) was calculated. FA composition was analyzed in mouse plasma and hepatic TG by gas chromatography–mass spectrometry (GC-MS). Glucose tolerance test (GTT) was performed as described [3].

Statistical analyses

Values are presented as mean $\pm$ SEM. GraphPad Prism 5.04 or Instat 3 (GraphPad Software Inc, San Diego, CA) was used for statistical analyses. One-way ANOVA followed by Bonferroni multiple comparison test was performed to determine significance. All differences between groups were based on mean values, but were only considered significant when $P < 0.05$.

Results

Physical characteristics of mice

Mice on HFD were more obese and insulin resistant than mice on ND [2]. Switching from HFD to ND induced a gradual weight reduction in WL-ND mice (Fig. 1a). To reach the same reduced body weight in WL-HFDr mice required limiting HFD intake (Fig. 1b). Of note, to keep the same body weight as in WL-ND mice, lower calorie intake was also required in WL-HFDr mice than in WL-ND mice (Fig. 1b). At 4 weeks after diet interventions, WL-ND and WL-HFDr mice had lower body weights, smaller perigonadal fat pads and livers (Supplemental Fig. I), and lower fat mass than HFC mice (Fig. 1c). Although body weight and weight of perigonadal fat pads and livers were comparable between WL-ND and WL-HFDr mice (Supplemental Fig. I), WL-ND mice had less total fat mass than WL-HFDr mice (Fig. 1c).

Effects of diet intervention on hepatic steatosis

HFC mice had severely fatty liver as indicated by numerous lipid droplets in liver sections and high amount of hepatic TGs (Fig 2a, 2b). Weight loss in both WL-ND and WL-HFDr mice reduced hepatic TG levels compared with those in HFC mice. However, WL-ND mice showed much greater improvement in hepatic steatosis (Fig. 2a) and had lower TG levels in the liver (Fig. 2b) and greater reductions in plasma levels of ALT, AST, and LDH (Supplemental Fig. II), indicating greater improvement in obesity-associated liver damage, than WL-HFDr mice.

mRNA levels of sterol regulatory element binding protein-1c (SREBP-1c), its targets ACC, FAS, SCD-1, and Elovl6, and CD36 were higher in the liver of HFC mice than NDC mice (Fig. 2c). Along with reduced hepatic TGs, both WL-ND and WL-HFDr mice showed decreased mRNA levels of SREBP-1c, and decreased or a trend towards decreased mRNA levels of ACC, FAS, SCD-1, and Elovl6 in the liver compared with HFC mice (Fig. 2c). WL-ND mice, with greater improvement in hepatic steatosis, also had lower mRNA levels of SCD-1 and a trend toward lower SREBP-1c, ACC, Elovl6, and CD36 levels in the liver than WL-HFDr mice (Fig. 2c). The C18:1/C18:0 ratio, which is known as the “desaturation index” and can be used to estimate SCD-1 activity [17], was higher in hepatic TG and plasma of HFC mice than NDC mice and significantly reduced in WL-ND but not in WL-HFDr mice (Supplemental Fig. III).

These data suggest that weight loss with ND resulted in greater improvement in obesity-associated hepatic steatosis and greater reduction in expression of hepatic lipogenic molecules than weight loss with HFD restriction.

Effect of diet intervention on hepatic inflammation

Chemokines such as CCL5 (RANTES), CCL2 (MCP-1), CCL4 (MIP-1 β), and CXCL14 (BRAX) were all higher in the livers of HFC mice than those of NDC mice (Fig. 3a). Compared with HFC mice, WL-ND mice displayed significant reductions in hepatic mRNA levels of RANTES, MCP-1, MIP-1 β , and BRAX, whereas WL-HFDr mice only showed a trend toward lower hepatic levels of MCP-1 and MIP-1 β . WL-ND mice had significantly

lower hepatic levels of RANTES, MCP-1, MIP-1 β , and BRAK than did WL-HFDr mice (Fig. 3a). mRNA levels of other chemokines, including CCL11 (eotaxin) and MIP-2, were low in the liver of all 4 groups of mice (data not shown). Therefore, we did not make comparisons for the hepatic levels of these chemokines among the various groups of mice.

HFC mice had higher hepatic mRNA levels of CD3 (a marker for total T cells), TCR α (an $\alpha\beta$ T cell marker) (Supplemental Fig. IV), F4/80 (a marker for total macrophages/DCs), and CD11c (a marker for activated macrophages or DCs [3,8]) than did NDC mice (Fig. 3b), suggesting that obese mice had increased T cells and macrophages/DCs in the liver compared with lean mice. WL-ND mice tended to have lower hepatic levels of CD3 and TCR α than HFC or WL-HFDr mice (Supplemental Fig. IV). Both WL-ND and WL-HFDr mice showed a trend toward lower hepatic F4/80 levels than in HFC mice, with no difference observed between WL-ND and WL-HFDr mice (Fig. 3b). However, WL-ND, but not WL-HFDr, mice had lower CD11c mRNA in the liver than did HFC mice. WL-ND mice also had lower hepatic CD11c mRNA than WL-HFDr mice (Fig. 3b).

These data indicate that weight loss with ND, but not with HFD restriction, significantly decreased hepatic inflammation in obese mice.

Effect of diet intervention on AT inflammation

Levels of chemokines, including MCP-1, MIP-2, RANTES, eotaxin, and MIP-1 β , and leukocyte markers, including CD3 and F4/80, were higher in AT of HFC than that of NDC mice (Fig. 4a and Supplemental Fig. Va and Vb). Weight loss in WL-ND mice tended to reduce MCP-1 and MIP-2 mRNA in AT compared to HFC mice (Fig. 4a). Unexpectedly, WL-HFDr mice showed significantly reduced MCP-1 and MIP-2 mRNA in AT compared with that of HFC mice and had a trend toward even lower AT MCP-1 and MIP-2 mRNA than WL-ND mice (Fig. 4a). In contrast, weight loss did not significantly reduce RANTES, eotaxin, or MIP-1 β mRNA in AT of either WL-ND or WL-HFDr mice compared with that of HFC mice (Supplemental Fig. Va).

Weight loss in both WL-ND and WL-HFDr mice tended to lower AT CD3 mRNA compared with HFC mice (Supplemental Fig. Vb). WL-HFDr mice also showed a trend toward lower AT F4/80 mRNA than HFC mice (Supplemental Fig. Vb). Flow cytometry confirmed that both WL-ND and WL-HFDr mice tended to have fewer T cells in AT than did HFC mice (Fig. 4b). Total F4/80⁺/CD11b⁺ cells did not change significantly in AT after weight loss in either WL-ND or WL-HFDr mice (data not shown). However, the proportion of CD11c⁺/CD11b⁺ cells (Supplemental Fig. VI), a subset of F4/80⁺/CD11b⁺ macrophages/DCs with proinflammatory properties in obese mouse AT [3,8], was significantly decreased in WL-HFDr mice and tended to be decreased in WL-ND mice compared with HFC mice (Fig. 4b). WL-HFDr mice also showed a trend toward lower CD11c⁺/CD11b⁺ cells in AT than WL-ND mice (Fig. 4b).

Adiponectin levels were lower and leptin levels were higher in AT of HFC mice than of NDC mice (Fig. 4c) [18]. Compared with those in HFC mice, AT adiponectin levels were significantly increased and leptin levels were significantly decreased in WL-ND mice; WL-HFDr mice showed a trend toward these changes, but had lower adiponectin levels and higher leptin levels than WL-ND mice (Fig. 4c). Plasma adiponectin levels showed similar changes as those in AT (Supplemental Fig. VII).

These data indicate that weight loss with ND increased adiponectin, decreased leptin, and tended to reduce chemokines and CD11c⁺/CD11b⁺ cells in AT, whereas weight loss with HFD (calorie) restriction tended to increase adiponectin and significantly decreased AT chemokines and CD11c⁺/CD11b⁺ cells.

Effect of diet intervention on plasma levels of MCP-1 and blood monocytes

HFC mice had higher plasma MCP-1 levels than did NDC mice. Both WL-ND and WL-HFDr mice showed decreased plasma MCP-1 levels compared with HFC mice, with lower plasma MCP-1 levels in WL-ND mice than in WL-HFDr mice (Fig. 5a). Blood CD11c⁺ monocytes, a subset of mouse monocytes that contributes to atherogenesis [3,16], were decreased in WL-ND, but not WL-HFDr, mice compared with HFC mice, and were lower in WL-ND than in WL-HFDr mice (Fig. 5b).

Effect of diet intervention on metabolic parameters

Obese mice were insulin resistant as indicated by higher plasma levels of insulin and glucose and higher HOMA-IR in HFC mice than in NDC mice (Supplemental Table I). Weight loss in WL-ND and WL-HFDr mice did not significantly lower plasma glucose or insulin levels or HOMA-IR (Supplemental Table I). However, GTT indicated that both WL-ND and WL-HFDr mice had improved glucose clearance compared with HFC mice, with no significant differences observed between WL-ND and WL-HFDr mice (Fig. 5c).

HFC mice had higher plasma cholesterol level than NDC mice (Supplemental Table I). Both WL-ND and WL-HFDr mice had lower plasma cholesterol level than HFC mice, with lower plasma cholesterol level in WL-ND than WL-HFDr mice (Supplemental Table I). Plasma TG level tended to be higher in HFC than NDC mice, was significantly reduced in WL-HFDr mice, and tended to be reduced in WL-ND mice (Supplemental Table I).

Effect of diet intervention on plasma FFAs

HFD and ND were different not only in total fat content but also in fat composition, including saturated FAs and PUFAs [2]. Saturated FAs and PUFAs have different effects on insulin resistance and inflammation [12,13]. Therefore, total FFAs and various FAs were examined in mouse plasma. HFC mice had higher plasma levels of total FFAs than did NDC mice. Weight loss in both WL-ND and WL-HFDr mice decreased plasma total FFA levels (Supplemental Table II). Although no significant differences were observed in plasma total FFAs between WL-ND and WL-HFDr mice, the plasma FFA profile was different in WL-ND and WL-HFDr mice, with lower levels of C12:0, C14:0, and C18:1, but higher levels of EPA and C18:2, in WL-ND than WL-HFDr mice (Supplemental Table II).

Discussion

In the present study, we found that in obese mice, the same degree of weight loss with ND ad libitum or with restriction of HFD resulted in differential effects on hepatic steatosis, hepatic and AT inflammation, and proportions of blood CD11c⁺ monocytes. Weight loss with ND, but not with HFD restriction, markedly reduced hepatic steatosis and inflammation, and normalized adiponectin levels and blood monocyte proportions. Unexpectedly, we also observed for the first time that weight loss with calorie restriction of HFD tended to decrease AT chemokines and CD11c⁺ cells more than weight loss with ND ad libitum. Therefore, compared with previous weight loss studies [3,10,14,15,19,20], our current study provided novel information on body weight-independent effects of weight loss with a low-fat ND vs. restriction of HFD on AT and liver inflammation, hepatic steatosis, and blood monocytes.

Despite the same degree of weight loss and comparable body weight, mice with ND-induced weight loss had less fat mass than those with HFD restriction-induced weight loss. The low fat content of the ND was most likely the major contributor to this difference. Another possible mechanism was that EPA and DHA, present in the ND only, have been shown to reduce fat mass [21]. Less dietary fat consumption may also have contributed to the greater

improvement in hepatic steatosis in ND-induced weight loss. Greater reduction in hepatic lipogenesis as indicated by lower levels of lipogenic molecules may be another contributor to the lower hepatic TGs in mice with ND-induced weight loss than in mice with HFD restriction-induced weight loss. Dietary PUFAs have been shown to suppress hepatic expression of SREBP-1 and its target genes, such as *FAS* and *SCD1* [22]. Therefore, inclusion of EPA and DHA in the ND may have contributed to the lower hepatic levels of lipogenic genes, particularly *SCD1*, in ND-induced weight loss. Our current study supports a beneficial effect of *SCD1* inhibition through dietary PUFA supplementation in improving obesity-associated hepatic steatosis.

Weight loss with ND, but not HFD restriction, ameliorated hepatic inflammation as indicated by lower hepatic levels of chemokines and CD11c, a marker for activated macrophages or DCs [3]. However, the hepatic levels of F4/80, a marker for total macrophages/DCs, were not significantly different among obese controls, mice with ND-induced weight loss, and mice with HFD restriction-induced weight loss. These data suggest plastic attributes of hepatic macrophages/DCs. Weight loss with ND, but not HFD restriction, resulted in conversion of these hepatic macrophages/DCs from “proinflammatory” phenotypes, with high expression of chemokines and CD11c, to a “resting” state, with reduction in chemokine and CD11c expression. Considering the proinflammatory properties of fat, particularly saturated fat [13,23], we postulate that the lower hepatic fat content may play important roles in conversion of hepatic macrophage/DC phenotypes and resolution of hepatic inflammation in ND-induced weight loss. In addition, higher levels of adiponectin, a molecule with anti-inflammatory properties [24], may also contribute to these effects of ND-induced weight loss.

Obesity and increased fat mass are associated with AT inflammation [2–5]. Because of lower fat mass with ND-induced weight loss, we had predicted that ND would reduce AT inflammation more than HFD restriction. Unexpectedly, we found that weight loss with HFD restriction tended to decrease AT MCP-1 and MIP-2 levels and CD11c⁺ cells more than weight loss with ND. On the other hand, weight loss with ND, but not HFD restriction, normalized adiponectin levels. These data indicated that AT adiponectin expression was regulated by pathways different from those for regulation of other AT inflammatory markers, and factors other than adiponectin may play important roles in regulation of chemokine expression and CD11c⁺ cells in AT of these mouse models.

To achieve the same body weight in both weight loss groups required not only restricting the amount of HFD consumed in the WL-HFDr group but also reducing the calorie intake below that of the WL-ND group. This is most likely due to the fact that animals on HFD are able to store the energy derived from dietary fat more efficiently in AT, thereby maintaining their body weight, than mice on a low-fat ND. As we provided HFD to the mice with HFD restriction-induced weight loss once a day, we observed that these mice consumed the HFD quickly and then had a long fasting period until the next HFD provision. Kosteli et al recently reported an increase in AT macrophage recruitment initially (days 3–7) during caloric restriction of HFD in obese mice, probably due to increased lipolysis induced by caloric restriction (fasting) [25]. They also found reduced AT macrophages at a later stage of caloric restriction (day 21 and afterwards) [25]. We analyzed AT macrophages on day 28 after dietary intervention, in samples collected after all the groups of mice had fasted for the same period of time; therefore, we did not expect significant confounding effects of an “acute” fasting-induced lipolysis on AT macrophages. Furthermore, the comparable plasma levels of total FFA in WL-ND and HFDr mice did not support a potentially confounding effect of lipolysis on AT macrophages in our mouse models. In contrast, our data suggested that low calorie intake and possibly periods of “chronic” fasting may be important in regulation of AT chemokines and CD11c⁺ cells, whereas type of diet may be more

important in regulation of AT adiponectin (and leptin) expression. Because dietary EPA and DHA induce adiponectin expression [26], inclusion of EPA and DHA in ND may play important roles in raising AT adiponectin. However, the favorable effects of EPA and DHA on other AT inflammatory markers [27] may be counteracted by the greater calorie intake in mice with ND-induced weight loss than in mice with HFD restriction-induced weight loss.

Compared to HFD restriction-induced weight loss, weight loss with ND resulted in greater reductions in blood CD11c⁺ monocytes. Based on the role of CD11c⁺ monocytes in atherogenesis [16], the reduction in CD11c⁺ monocytes would favor protective effects against obesity-associated atherosclerosis. MCP-1 increases CD11c expression on monocytes [28]. The lower plasma MCP-1 levels in ND-induced weight loss may have contributed to the lower blood CD11c⁺ monocytes. Recent studies indicated that saturated FAs induced monocyte inflammation [29]. Mice with ND-induced weight loss had lower levels of saturated FA, but higher levels of PUFAs, than mice with HFD restriction-induced weight loss. The potential effects of various types of FAs and also adiponectin on monocyte CD11c expression warrant further investigation.

The comparable improvements in glucose clearance as assessed by GTT between ND- and HFD restriction-induced weight loss may be explained by the dissociation of hepatic steatosis and insulin resistance [30] and a trend toward higher AT inflammatory markers with ND-induced weight loss than HFD restriction-induced weight loss.

Li et al reported that switching from HFD to ND for 3 weeks improved insulin sensitivity and ameliorated hepatic steatosis and inflammation in obese mice [20], which was consistent with our observation in WL-ND mice. However, they observed more pronounced reduction in AT inflammatory markers in their ND-induced weight loss group than we did in our WL-ND mice. Kalupahana et al reported that HFD restriction for 2 months in obese mice reduced AT inflammation, including decreased MCP-1, reduced plasma triglyceride levels, and improved hepatic steatosis [19], observations consistent with ours in WL-HFD mice. However, in contrast to our observations, they found that obese mice with HFD restriction maintained high levels of FFAs in plasma but had significantly decreased plasma insulin levels and HOMA-IR [19]. Differences in types of diets and periods of dietary interventions may have contributed to these discrepancies. Whereas these studies examined the effect of either ND-induced weight loss alone [20] or HFD restriction-induced weight loss alone [19], we are the first to report the differential effects of ND- and HFD restriction-induced weight loss on inflammation in mice with diet-induced obesity.

In summary, using a mouse model of HFD-induced obesity, we demonstrated differential effects of ND- and HFD restriction-induced weight loss. In particular, weight loss with ND, but not with HFD restriction, markedly alleviated hepatic steatosis and inflammation as assessed by hepatic TGs and chemokine and CD11c expression. ND-induced weight loss normalized adiponectin expression in AT and tended to attenuate AT expression of chemokines and CD11c, whereas HFD restriction-induced weight loss significantly attenuated AT expression of chemokines and CD11c and tended to increase AT adiponectin. Weight loss with ND, but not with HFD restriction, normalized blood CD11c⁺ monocytes. Although improvements in glucose tolerance and insulin resistance were not significantly different between ND- and HFD restriction-induced weight loss in this short-term study, we postulate that long-term weight loss with ND would be more beneficial considering its greater ability to alleviate hepatic inflammation and steatosis, a potentially important contributor to obesity-linked insulin resistance and a risk factor for hepatic cirrhosis and carcinoma [9], and to normalize blood CD11c⁺ monocytes, a contributor to atherosclerosis [16]. Also, given the fact that HFD calorie restriction-induced weight loss tended to reduce AT inflammation more than ND-induced weight loss, we propose that long-term weight loss

with low-fat ND along with calorie restriction would result in the most beneficial effects in obesity treatment.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Figure 1. Physical characteristics of mice

Daily (a) body weight and (b) diet intake during the comparative diet interventions. Daily calorie intake was calculated based on the amount of daily diet intake and energy values from each diet. (c) Total fat mass at 2 days before the end of diet interventions. HFC, obese control mice; NDC, lean control mice; WL-ND, obese mice with ND-induced weight loss; WL-HFDr, obese mice with HFD restriction-induced weight loss. n=7–19 mice/group.

Figure 2. Effect of diet interventions on hepatic steatosis

At 4 weeks after comparative diet interventions, livers were harvested from mice. (a) Representative hematoxylin and eosin (H&E) staining of liver samples, from at least 3 mice in each group with similar staining. Magnification $\times 100$. (b) TG content in the liver measured in liver lipid extract. $n=7-10$ mice/group. (c) mRNA levels of lipogenic molecules in the liver examined by qRT-PCR. $n=3$ samples/group; each sample was pooled from 3 mice.

a. Chemokine expression in the liver

*P<0.05, **P<0.01 vs. HFC

b. Macrophage/DC markers in the liver

*P<0.05, **P<0.01 vs. HFC

Figure 3. Effect of diet interventions on liver inflammation

(a) mRNA levels of chemokines in the liver determined by RPA (for RANTES, MCP-1, and MIP-1 β) or qRT-PCR (for BRAK). n=3–5 samples/group. (b) mRNA levels of markers for macrophages/DCs (F4/80 and CD11c) in the liver determined by RPA (for F4/80) or qRT-PCR (for CD11c). n=3–9 samples/group.

a. Chemokine expression in AT

*P<0.05, **P<0.01 vs. HFC

b. T cells and macrophages/DCs in AT

**P<0.01 vs. HFC

c. Adiponectin and leptin in AT

Figure 4. Effect of diet interventions on AT inflammation

(a) mRNA levels of chemokines in AT determined by RPA. n=9–10 mice/group. (b) T cells and CD11c⁺/CD11b⁺ cells in S/Vs of mouse AT examined by flow cytometry. n=6–8 samples/group. (c) mRNA levels of adiponectin and leptin in AT examined by qRT-PCR. n=4–12 mice/group.

Figure 5. Effect of diet interventions on plasma levels of MCP-1, blood CD11c⁺ monocytes, and glucose tolerance

(a) Plasma levels of MCP-1 measured using ELISA. n=9 mice/group. (b) Blood CD11c⁺ monocytes examined by flow cytometry. n=13–14 mice/group. (c) Glucose tolerance test. n=4–5 mice/group.