Dietary fatty acids modulate antigen presentation to hepatic NKT cells in nonalcoholic fatty liver disease[S]

Jing Hua,* Xiong Ma,* Tonya Webb,[†] James J. Potter,[§] Mathias Oelke,[†] and Zhiping Li[1,§]

Shanghai Renji Hospital,* Jiaotong University School of Medicine, Shanghai Institute of Digestive Disease, Shanghai, China; and Departments of Medicine[§] and Pathology,[†] Johns Hopkins University, Baltimore, MD

Abstract Dietary fatty acids are major contributors to the development and progression of insulin resistance and nonalcoholic fatty liver disease (NAFLD). Dietary fatty acids also alter hepatic NKT cells that are activated by antigens presented by CD1d. In the current study, we examine the mechanism of dietary fatty acid induced hepatic NKT cell deficiency and its causal relationship to insulin resistance and NAFLD. We discover that dietary saturated fatty acids (SFA) or monounsaturated fatty acids (MUFA), but not polyunsaturated fatty acids (PUFA), cause hepatic NKT cell depletion with increased apoptosis. Dietary SFA or MUFA also impair hepatocyte presentation of endogenous, but not exogenous, antigen to NKT cells, indicating alterations of the endogenous antigen processing or presenting pathway. In vitro treatment of normal hepatocytes with fatty acids also demonstrates impaired ability of CD1d to present endogenous antigen by dietary fatty acids. Furthermore, dietary SFA and MUFA activate the NFκB signaling pathway and lead to insulin resistance and hepatic steatosis. In conclusion, both dietary SFA and MUFA alter endogenous antigen presentation to hepatic NKT cells and contribute to NKT cell depletion, leading to further activation of inflammatory signaling, insulin resistance, and hepatic steatosis.— Hua, J., X. Ma, T. Webb, J. J. Potter, M. Oelke, and Z. Li. **Dietary fatty acids modulate antigen presentation to hepatic NKT cells in nonalcoholic fatty liver disease. *J. Lipid Res.* 2010.** 51: **1696–1703.**

Supplementary key words inflammation • obesity • insulin resistance

Dietary fatty acids have been shown to influence immune function (1). Polyunsaturated fatty acids (PUFA) can modulate cytokine production, lymphocyte proliferation, and apoptosis (2, 3). Saturated fatty acids (SFA) affect MHC I-mediated antigen presentation, impair immune response, and increase host susceptibility to infection (4, 5). Dietary fatty acids are also the major contributing factor to metabolic syndrome, coincidence conditions of obesity, insulin resistance, hypertension, dyslipidemia, and fatty liver diseases (6). Dietary SFAs induce both obesity and insulin resistance in experimental animals and humans (7, 8). These observations provide the basis for the hypotheses that altered immune function by dietary fatty acids may contribute to the development of metabolic syndrome. In fact, inflammatory conditions are well linked to insulin resistance, obesity, and fatty liver diseases (9, 10). Human population studies have also linked insulin resistance to systemic inflammation (11, 12). In liver, natural killer T (NKT) cells are the key mediator between inflammatory condition and hepatic insulin resistance and steatosis (6, 13).

NKT cells are a group of "unconventional" T cells that express markers characteristic for both natural killer (NK) cells and T cells. They are most abundant in the liver and can balance the production of pro-inflammatory and anti-inflammatory cytokines (14, 15). Our previous studies have shown that high-fat diets induce hepatic NKT cell depletion and lead to local and systematic inflammatory conditions that contribute to insulin resistance and fatty liver diseases (6). However, there is little knowledge about the mechanism by which dietary fatty acids regulate hepatic NKT cells. An earlier study has shown that imbalance in lipid metabolism can alter lipid antigen presentation to NKT cells (16).

In the current study, we evaluated the effect of different kinds of dietary fatty acids on hepatic NKT cells. Importantly, we investigated mechanisms underlying the immuno-modulatory function of dietary fatty acids and their role in the inflammatory process, insulin resistance,

This work was supported by National Institutes of Health/National Institute of Diabetes and Digestive and Kidney Diseases Grant R01 DK-075990 (Z.L.) and by National Natural Science Foundation of China Grant 30971331 (J.H.). Its contents are solely the responsibility of the authors and do not necessarily represent the official views of the National Institutes of Health or other granting agencies.

Manuscript received 7 October 2009 and in revised form 24 January 2010.

Published, JLR Papers in Press, February 24, 2010
DOI 10.1194/jlr.M003004

Abbreviations: DHA, docosahexaenoic acid; GalCer, galactosylceramide; IFN, interferon; IL, interleukin; MUFA, monounsaturated fatty acid; NAFLD, nonalcoholic fatty liver disease; NFκB, nuclear factor-κB; NKT, natural killer T; OS, oleic acid; PA, palmitoleic acid; PUFA, polyunsaturated fatty acid; SFA, saturated fatty acid.

[1]To whom correspondence should be addressed.
e- mail: zhipingli@jhmi.edu

[S] The online version of this article (available at http://www.jlr.org) contains supplementary data in the form of one figure.

and hepatic steatosis. Results from our current study provide a better understanding of the relation between nutrition and immune function.

METHODS

Animal experiments

Adult (age 6–8 weeks) male wild-type C57BL/6 mice were purchased from Jackson Laboratories (Bar Harbor, ME). The mice were divided into five groups and fed a custom-made commercial diet enriched with certain fatty acids (BioServ, Inc., Frenchtown, NJ), a custom-made commercial diet enriched with mixed fatty acids(BioServ), or a normal diet (**Table 1**) for 12 weeks. The SFA-rich diet contained lauric (31·1%), myristic (27·8%), palmitic (14·0%), oleic (11·6%), and linolenic (11·0%) acids with <5% palmitoleic and stearic acids. The MUFA-enriched olive-oil diet contained palmitic (12·5%), oleic (71·3%), and linolenic (9·9%) acids with <5% myristic, palmitoleic, and stearic acids. The PUFA-enriched fish-oil diet contained docosahexaenoic (22.3%), linoleic (14.7%), palmitic (20.6%), and myristic (16.3%) acids with <5% stearic, arachidic, and erucic acids. All mice were maintained in a temperature- and light-controlled facility and permitted to consume water ad libitum. All animal experiments fulfilled National Institutes of Health (NIH) and Johns Hopkins University criteria for the humane treatment of laboratory animals.

Hepatocyte and NKT cell isolation

Hepatic mononuclear cells (HMNC) were isolated as previously described(6). HMNCs were then labeled with CD1d tetramers (NIH tetramer facility) loaded with a ligand (PBS-57, an analog of α-galactosylceramide (GalCer) or anti-mouse fluorescent antibodies against CD8, CD4, CD3, and NK1.1 (Pharmingen, San Diego, CA). After surface labeling, HMNCs were analyzed by flow cytometry (Becton Dickinson, Palo Alto, CA), and NKT cells ($NK1.1^+$, $CD3^+$) were isolated using a FACSVantage SE high speed sorter (Becton Dickenson). Hepatocytes were isolated by in situ perfusion of the liver then cultured on collagen I-coated plates as described previously(17).

Intracellular cytokine labeling of liver NKT cells

For intracellular staining of cytokines, we utilized an Intracellular Cytokine Staining Kit (Pharmingen). Briefly, HMNCs were incubated with a leukocyte activation cocktail, which includes phorbol 1,2-myristate 1,3-acetate (PMA, 50 ng/ml), ionomycin (500 ng/ml), and GolgiPlug (1 μl/ml). Cells were labeled with CD1d tetramers as described above and then permeabilized with Cytoperm/Cytofix (Pharmingen) according to the manufacturer's instructions. After permeabilization, the cells were further labeled with anti-mouse interleukin (IL)-4 and interferon (IFN)γ (Pharmingen), and then evaluated by flow cytometry.

In vitro NKT cell activation and proliferation assays

$Vα14^+$ mouse CD1d-specific NKT hybridoma cells (DN32.D3) were cocultured with a mouse fibroblast cell line (LCD1dwt) that has been stably transfected with CD1d and naturally expresses an endogenous NKT cell ligand (18), in the presence or absence of FFAs, such as palmitoleic acid (PA), oleic acid (OA), and docosahexaenoic acid (DHA) (Sigma-Aldrich, St Louis, MO). IL-2 released from NKT cells to the media were determined by commercially available, mouse-compatible ELISAs (R and D systems, Inc., Minneapolis, MN) with recombinant murine cytokines as standards. For the NKT cell proliferation assay, freshly isolated primary liver NKT cells were incubated with CD1d-Ig based artificial antigen presenting cells (aAPC) as previously described (19) in the presence or absence of FFAs for 12 days. The number of viable NKT cells was determined by trypan blue exclusion.

Hepatocytes antigen presentation to NKT cells

After isolation, some hepatocytes were loaded with α-GalCer (100ng/ml, ALEXIS Biochemical, San Diego, CA) for 4 h and washed extensively before coculture with NKT hybridoma cells. Other untreated hepatocytes were used to determine

TABLE 1. Diet composition

	Normal Diet	High Fat Control Diet (Bio-serv F3282)	High Saturated Fatty Acid Diet (Bio-serv F5423)	High Polyunsaturated Fatty Acid Diet (Bio-serv F5424)	High Monounsaturated Fatty Acid Diet (Bio-serv F5425)
Ingredient (g/kg)					
Casein	228.0	228.0	228.0	228.0	228.0
Cornstarch	500.0	220.0	220.0	220.0	220.0
Fiber	50	—	—	—	—
Sucrose	118.7	140.2	140.2	140.2	140.2
AIN mineral mix 76A	40	40	40	40	40
AIN vitamin mix 76A	10	10	10	10	10
DL-methionine	2.0	2.0	2.0	2.0	2.0
Choline bitartrate	1.3	1.3	1.3	1.3	1.3
Corn oil	50	—	53	53	—
Lard	—	358.5	—	—	—
Coconut oil	—	—	305.5	—	—
Fish oil	—	—	—	305.5	—
Olive oil	—	—	—	—	358.5
Calculated values (%)					
Protein	20	20	20	20	20
Fat	5	35.9	35.9	35.9	35.9
Fiber	5	—	—	—	—
Ash	3.2	3.2	3.2	3.2	3.2
Moisture	<5	<5	<5	<5	<5
Carbohydrate	61.8	36.3	36.3	36.3	36.3
Energy (kcal/g)	3.635	5.478	5.478	5.478	5.478

To maintain the same adequate amount of dietary EFA among all high-fat diets, a small amount (5.3%) of corn oil was added to SFA and PUFA diets. Each type of high-fat diet has the same total fat content (35.85%) and EFA composition (3.5%). Abbreviations: EFA, essential fatty acid; PUFA, polyunsaturated fatty acid; SFA, saturated fatty acid.

their endogenous lipid antigen presentation to NKT cells. IL-2 production from NKT hybridoma cells indicated NKT cell activation by hepatocytes. In separate experiments, freshly isolated hepatocytes from normal diet fed mice were first incubated with individual FFAs for 24 h. After extensive washing, these hepatocytes were then cocultured with NKT hybridoma cells.

Glucose tolerance test (GTT), liver histology and triglyceride content, RNA isolation, hepatic gene expression, Western blot, and DNA binding activity of NF-κB

All these experiments were performed as previously described(13).

Statistical analysis

All values are expressed as mean ± SE. Treatment related differences were evaluated by ANOVA. The paired-individual means were compared by *t*-test.

RESULTS

Dietary saturated and monounsaturated, but not polyunsaturated, fatty acids induce insulin resistance, hepatic steatosis, and NKT cell depletion

Our previous study showed that a high fat diet with mixed fatty acids (serving as the control high fat diet in the current study, HFC) causes obesity, hepatic steatosis and NKT cell depletion (6). Other studies have produced conflicting results concerning which kind of fatty acids induce obesity and insulin resistance (20, 21). In order to better understand which dietary fatty acid contributes to high fat diet-induced metabolic syndrome, we fed wild-type C57BL6 mice with custom made diets enriched with saturated fatty acids (SFA), or monounsaturated fatty acids (MUFA), or polyunsaturated fatty acids (PUFA) for 12 weeks. Each diet has a similar energy composition of fats, carbohydrates and proteins, but differs in the predominant fatty acid added (Table 1). The results show that SFA and MUFA diets, but not the PUFA diet, induce greater weight gain in wild-type mice, despite a similar total caloric intake among these animals (**Fig. 1A**). Mice fed SFA and MUFA diets also developed insulin resistance as reflected by glucose tolerance tests (Fig. 1B). Since non-alcoholic fatty liver disease (NAFLD) is closely associated with obesity and insulin resistance, we evaluated the liver from these mice for NAFLD. Mice fed SFA and MUFA diets had significant hepatic steatosis as seen in liver histology (Fig. 1C) as well as significantly increased hepatic triglyceride contents (Fig. 1D). Therefore, it appears that different dietary fatty acids impact differently on the high fat diet-induced obesity, insulin resistance and fatty liver disease. Next, we tried to identify the mechanism of which dietary fatty acids exert their effects.

It has been shown that the development of obesity, insulin resistance and hepatic steatosis is associated with selective hepatic NKT cell depletion. We examined hepatic NKT cell contents in animals fed different high fatty acid diets. Dietary SFA and MUFA, but not PUFA induced significant hepatic NKT cell depletion (**Fig. 2A**, B). Hepatic T cells are not affected by any kind of dietary fatty acids (Fig. 2B). As previous published (6), the total HMNC content is not influenced by high fat diets. Therefore the percentage of hepatic NKT cells collates their number in the liver. Hepatic NKT cells produce both the pro-inflammatory cytokine, IFN-γ, and the anti-inflammatory cytokine, IL-4. We examined the cytokine profile of remaining hepatic NKT cells after different dietary fatty acid treatment. Intracellular cytokine staining was performed in conjunction with cell surface marker labeling. Dietary MUFA causes a significant decrease in IL-4 expression, while dietary PUFA causes a significant decrease in IFNγ expression in NKT cells (Fig. 2C, D). Therefore, dietary fatty acids not only alter the hepatic NKT cell content but also impact the NKT cell cytokine profiles.

Dietary fatty acids impair hepatocyte presentation of endogenous antigen to NKT cells

NKT cells recognize endogenous and exogenous lipid antigens presented by the MHC class I like molecule, CD1d. It has previously been shown that hepatic CD1d down-regulation contributes to NKT cell depletion (22), thus we evaluated the hepatic expression of CD1d in animals fed different fatty acid-enriched diets. Overall, diets of high fatty acid content led to lower hepatic CD1d expression (**Fig. 3A**). However, none of the fatty acid enriched diets caused a statistically significant reduction of CD1d expression in hepatocytes (data not shown). We isolated hepatocytes from animals treated with different fatty acid diets and incubated these hepatocytes with the NKT cell hybridoma, DN32.D3. Hepatocytes from SFA and MUFA fed animals show a decreased ability to stimulate NKT cells, as reflected by decreased IL-2 production (Fig. 3B). When these hepatocytes were loaded with an exogenous ligand, α-GalCer, they show a similar ability to stimulate NKT cells regardless of whether they were from normal diet or high fat diet fed mice (Fig. 3C). The stimulation of NKT cells by hepatocytes is CD1d dependent because there was no effect by hepatocytes isolated from CD1d knockout (CD1dko) mice (Fig. 3B). These results indicate that diets enriched by SFA or MUFA impair hepatocyte presentation of endogenous antigen to NKT cells by either inhibiting the endogenous ligand or by interfering with the interaction between the endogenous ligand and CD1d. To further confirm the effect of fatty acids on hepatocyte presentation of endogenous ligand to NKT cells, we isolated normal hepatocytes from wild-type animals fed normal diet and treated them in vitro with PA, OA or DHA to represent the treatment of SFA, MUFA or PUFA, respectively. In vitro fatty acid treatment has little effect on hepatocyte expression of CD1d (**Fig. 4A**). However, in vitro SFA (PA) treatment does reduce the ability of hepatocytes to stimulate NKT cells (Fig. 4B), which can be restored by loading exogenous ligand α-GalCer (Fig. 4C). Our studies show that both in vivo and in vitro fatty acid treatment reduced the ability of hepatocytes to stimulate NKT cells.

Dietary fatty acids induce inflammatory signaling

As mentioned earlier, hepatic NKT cells can secrete both pro-inflammatory cytokines (IFN-γ) and anti-inflammatory

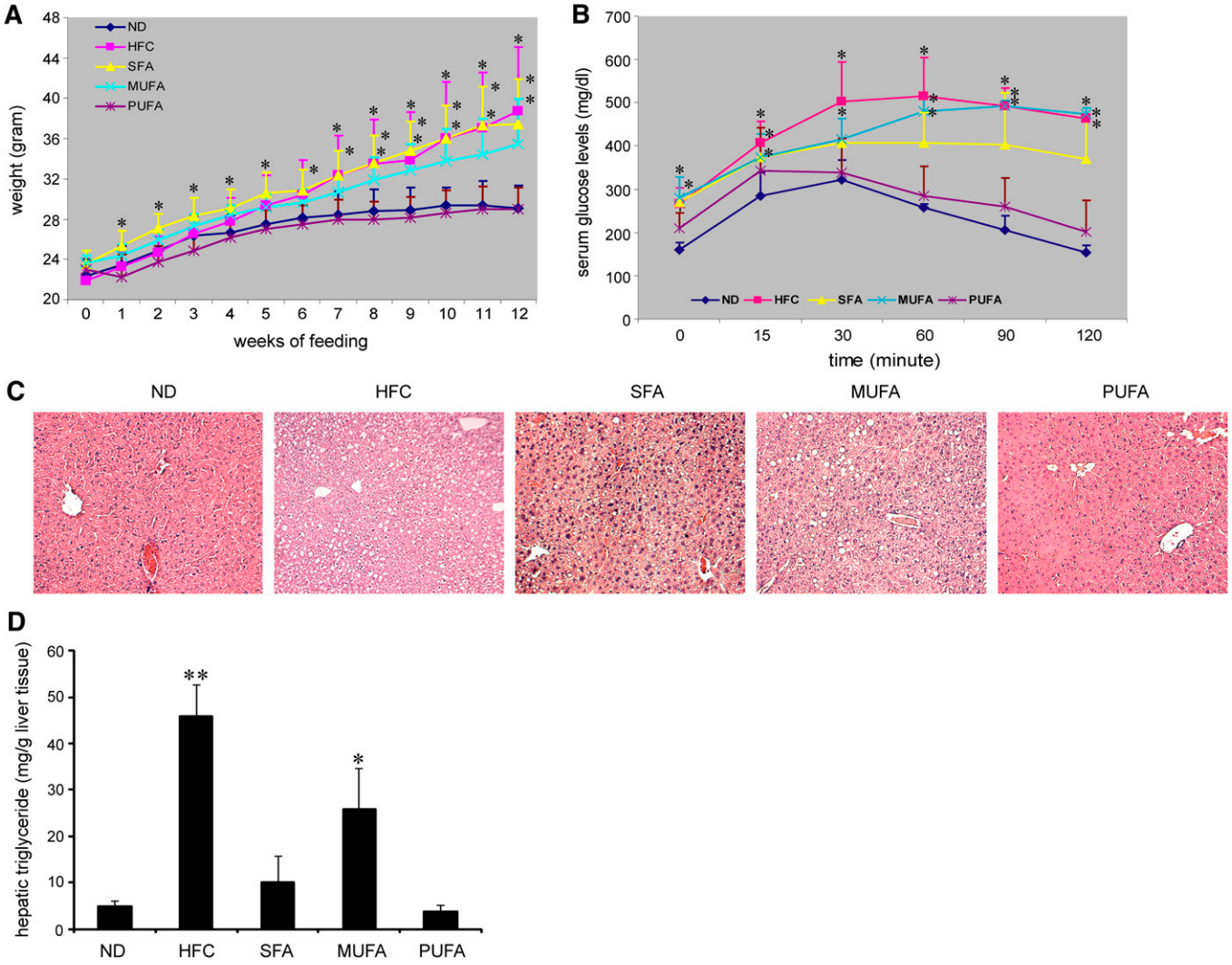

Fig. 1. SFA and MUFA diets, but not PUFA diet, cause obesity, insulin resistance, and fatty liver disease. Wild-type C57BL6 mice were fed diets enriched with certain fatty acids (see Table 1) for 12 weeks. A: animal weight, (B) glucose tolerance tests, (C) liver histology, and (D) hepatic triglyceride contents were performed. (*$P < 0.05$ versus ND) (n = 4/group). HFC, high fat control diet; MUFA, monounsaturated fatty acid; ND, normal diet; PUFA, polyunsaturated fatty acid; SFA, saturated fatty acid.

cytokines (IL-4). Also, they play an important role in regulating hepatic cytokine production and balancing inflammatory response. Hepatic NKT cell depletion is associated with excessive pro-inflammatory cytokine production and activation of inflammatory signaling (13). To evaluate the effect of fatty acid diets on hepatic inflammatory signaling, we examined the hepatic cytokine expression profile. SFA and MUFA, but not PUFA, diets significantly increased the expression of IFN-γ and decreased the expression of IL-4 (**Fig. 5A**), indicating a pro-inflammatory hepatic cytokine profile was induced by SFA and MUFA diets. These results represent the cytokine profile in the whole liver tissue, which is different from the cytokine profile of NKT cells shown in Fig. 2. In the liver, there are many other cells that also produce IFN-γ, such as NK cells. The cytokine profile of the whole liver is the sum of all cells, including NKT cells. IKK-β, which phosphorylates IκBα, is a key factor that mediates diet-induced obesity and insulin resistance (9). Therefore, the ratio of phospho-IκBα to total-IκBα represents IKK-β activity. SFA and MUFA diet–fed mice have significantly increased hepatic IKK-β activity, as indicated by an increased ratio of phospho-IκBα to total-IκBα (Fig. 5B). We further evaluated NFκB binding activities, the downstream signal for IκBα, with similar findings. SFA and MUFA diets increased NFκB binding activity, although only the MUFA diet group reached statistical significance (Fig. 5C). Because adipokines made by fat tissue also contribute greatly to the regulation of inflammation and insulin resistance, we evaluated the serum levels of leptin and adiponectin. SFA and MUFA diets significantly increased the serum level of leptin (Fig. 5D). This was consistent with the increased obesity of the animals on SFA or MUFA diets. No differences in the adiponectin level were observed among diet groups (Fig. 5D).

DISCUSSION

High dietary fat intake is known to be associated with obesity, insulin resistance, and NAFLD (6, 23, 24). However, the impact of dietary fat composition on the formation of

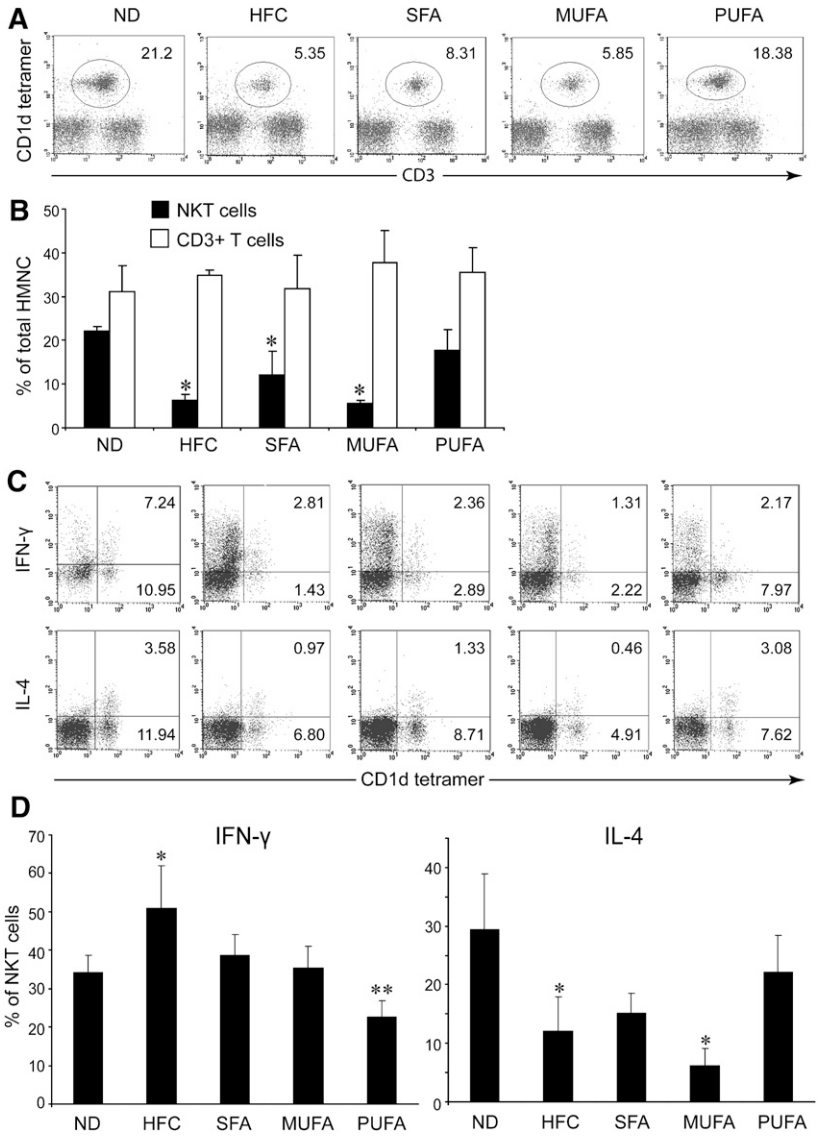

Fig. 2. SFA and MUFA diets selectively depleted hepatic NKT cells and alter NKT cell cytokine profile. Wild-type C57BL6 mice were fed diets enriched with certain fatty acids (see Table 1) for 12 weeks. Hepatic mononuclear cells (HMNC) were isolated and NKT cells were identified with CD1d tetramer loaded with a ligand and colabeled with IFN-γ, IL-4, and CD3. The total HMNC content was not influenced by high fat diets. Therefore, the percentage of hepatic NKT cells reflected their number in the liver. A: Representative dot plots of hepatic $CD3^+$ CD1d tetramer positive cells. B: Mean (± SD) results of hepatic NKT cell content and T-cell content. C: IFNγ and IL-4 intracellular staining of NKT cells. D: Mean (± SD) results of IFN-γ and IL-4 intracellular staining of NKT cells (gated on $CD3^+$ CD1d tetramer positive cells). (*$P < 0.05$ versus ND) (n = 4/group). IFN, interferon; IL, interleukin; MUFA, monounsaturated fatty acid; NDSFA, saturated fatty acid; NKT, natural killer T; PUFA, polyunsaturated fatty acid.

NAFLD is still poorly understood. In some studies, the dietary intake of high amounts of saturated fatty acids and low amounts of polyunsaturated fatty acids is linked to patients with NAFLD (20). Another study showed a high intake of n-6 fatty acids in patients with nonalcoholic steatohepatitis (NASH) (21). In our current study, we have shown that dietary saturated and monounsaturated fatty acids play a much more significant role in the pathogenesis of NAFLD than dietary polyunsaturated fatty acids. It also appears that a high-fat diet with mixed fatty acids (i.e., the high fat control diet in this study) may have synergistic effects on the formation of obesity, insulin resistance, and NAFLD. However, due to the difficulty in performing dose-saturation studies of dietary fatty acid composition, we cannot draw a definitive conclusion on these synergistic effects.

The current study confirms the previous finding that hepatic NKT cells are key contributors of high fat diet–induced hepatic steatosis (6, 13). SFA- or MUFA-induced fatty liver is associated with depletion of hepatic NKT cells. Dietary fatty acids also cause a significant alteration of NKT cell cytokine profile. Because NKT cells have many subgroups and each subgroup has a unique profile of cytokine production (25), these findings could result from modifications of NKT cell subgroups by different

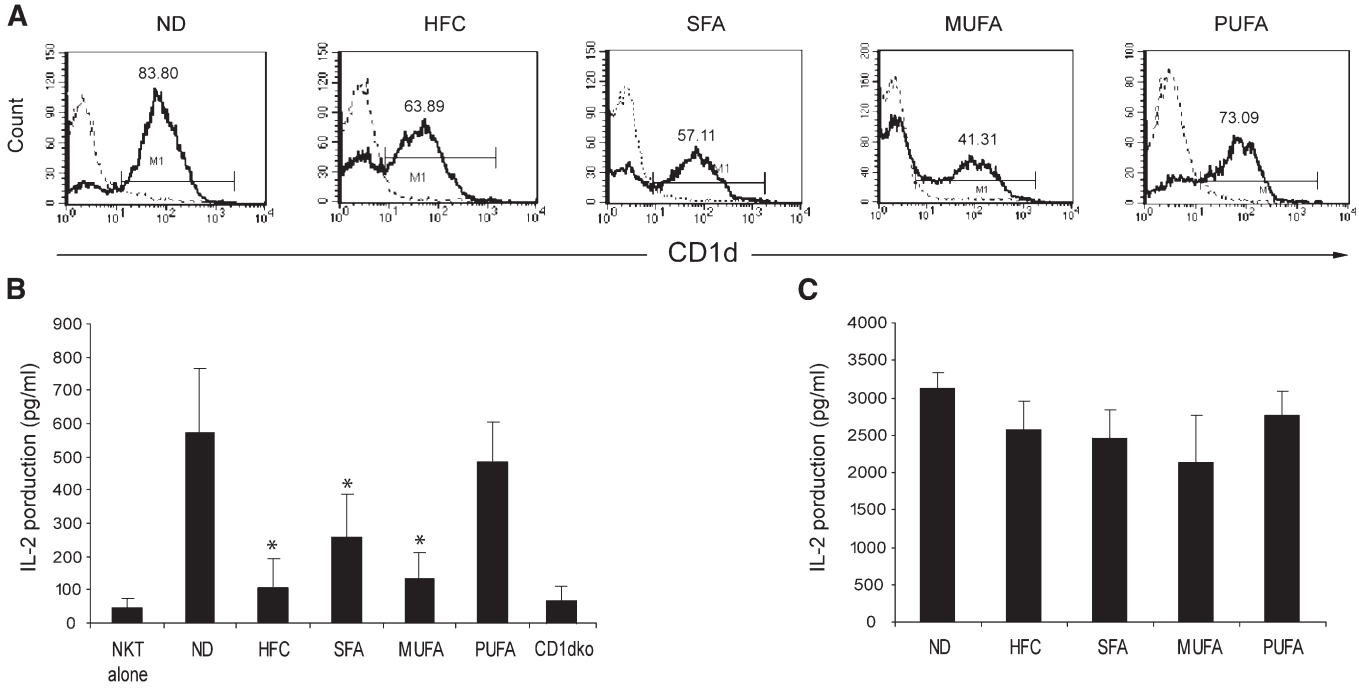

Fig. 3. Dietary fatty acids impair hepatocyte presentation of endogenous antigen to NKT cells in vivo. Wild-type C57BL6 mice were fed diets enriched with certain fatty acids (see Table 1) for 12 weeks. Hepatocytes were isolated and CD1d expression was determined by flow cytometry. A: Representative histogram of hepatocyte CD1d expression. Dotted line indicates staining with isotype control. B: Hepatocytes were isolated from either WT mice fed normal, SFA, MUFA, PUFA, or HFC diet, or from CD1dko mice and then cocultured with a NKT cell line. IL-2 released from NKT cells indicated activation of NKT cells by hepatocytes. (*$P < 0.05$ versus ND) (n = 3/group). C: Similar hepatocytes were isolated from WT mice and then preloaded with α-GalCer. After extensive washing, the hepatocytes were then cocultured with a NKT cell line. IL-2 released from NKT cells indicates activation of NKT cells by hepatocytes. (n = 3/group). CD1dko, CD1d knockout; GalCer, galactosylceramide; HFC, high fat control diet; MUFA, monounsaturated fatty acid; ND, normal diet; NKT, natural killer T; PUFA, polyunsaturated fatty acid; SFA, saturated fatty acid; WT, wild type.

dietary fatty acids. We are currently investigating the effect of dietary fatty acids on NKT cell subgroups.

The ability of certain fatty acids to influence the function of different components of the immune system has been recognized for many years (26). SFA and MUFA influence major histocompatibility complex (MHC) I–mediated antigen presentation (5). SFA causes a significant reduction in MHC class I surface expression and inhibits in the rate of antigen presenting cell (APC)-T cell conjugation. NKT cells recognize a group of glycolipid antigens bound to the MHC class I-like molecule CD1d, which is noncovalently associated with β2-microglobulin (27). Previous study shows that diets containing high saturated or unsaturated fatty acids result in similar alterations in the phospholipid class distribution and fatty acid composition in the liver (28). It is known that lipid accumulation due to dysregulation of lipid metabolism can cause defective presentation of lipid antigens to NKT cells and impacts NKT cell accumulation (16). Little is known, however, about the effect of dietary fatty acids on CD1d-mediated NKT cell activation.

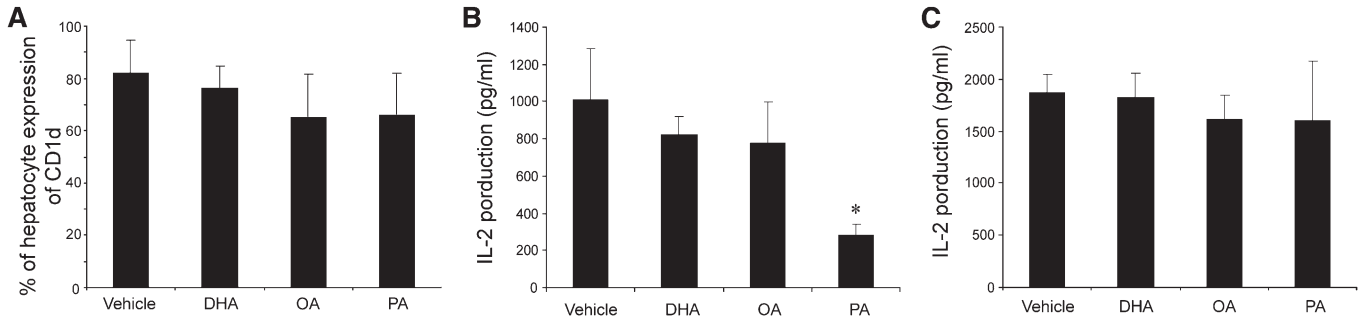

Fig. 4. Fatty acids impair hepatocyte presentation of endogenous antigen to NKT cells in vitro. Hepatocytes were isolated from wild-type C57BL6 mice fed normal diet and then treated with PA, OA, or DHA in culture. A: CD1d expression on these hepatocytes were determined by flow cytometry. B: After treatment with fatty acids, hepatocytes were cocultured with NKT cells. IL-2 released from NKT cells indicated activation of NKT cells by hepatocytes. (*$P < 0.05$ versus vehicle treated group) (n = 3/group). C: After treatment with fatty acids, hepatocytes were preloaded with α-GalCer. Following extensive washing, the hepatocytes were then cocultured with NKT cells. (n = 3/group). DHA, docosahexaenoic acid; GalCer, galactosylceramide; NKT, natural killer T; OS, oleic acid; PA, palmitoleic acid.

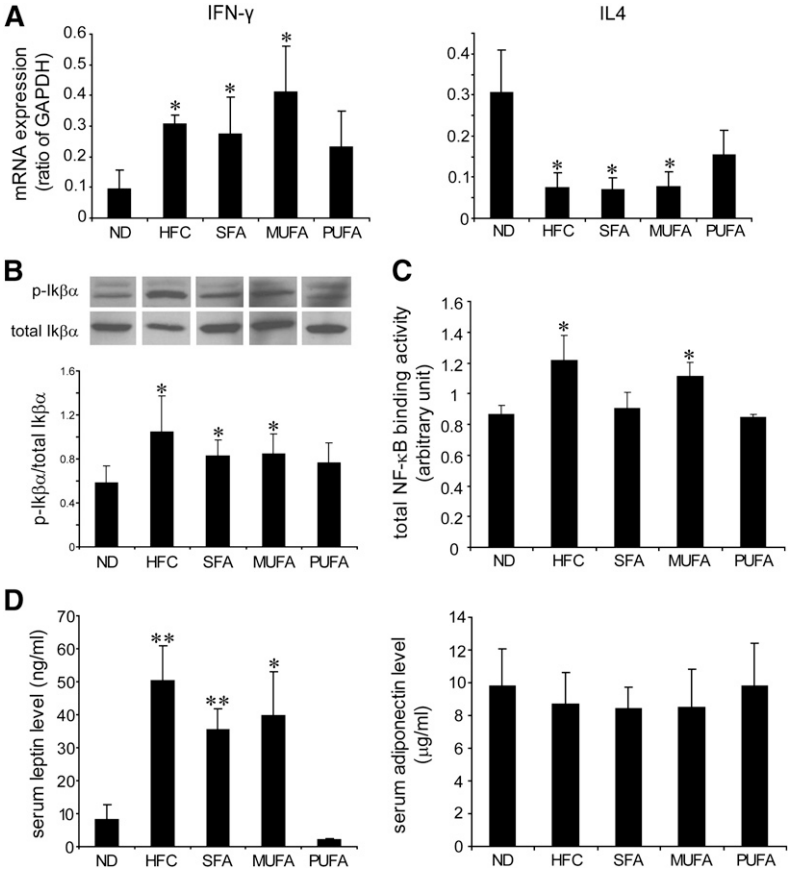

Fig. 5. The effects of dietary fatty acids on hepatic cytokine expression, inflammation signaling and serum adipokines. Wild-type C57BL6 mice were fed diets enriched with certain fatty acids (see Table 1) for 12 weeks. A: Hepatic IFNγ and IL-4 expressions were determined by quantitative real time PCR and normalized with GAPDH expression. B: The expression of total-IκBα in whole liver extract was measured by Western blot. The phospho-IκBα expression was then measured by stripping and reprobing the same membrane. Representative autoradiographs are shown. Mean (± SD) results from triplicate experiments are graphed. C: ELISA-based NF-κB binding activity assay. D: Serum levels of leptin and adiponectin. (*$P < 0.05$; **$P < 0.01$ versus ND) (n = 4/group). IFN, interferon; IL, interleukin; ND, normal diet.

In the current study, we have shown for the first time that high-fat diets impair the ability of CD1d to present endogenous antigen to NKT cells. This likely contributes to the mechanism of dietary fatty acid-induced hepatic NKT cell depletion. The exact endogenous ligand for NKT cells is still in debate (29, 30). Previous studies show that high-fat diets alter the composition of liver ceramides, which are likely the antigens for NKT cells (31). It will be interesting to investigate which candidate endogenous lipid has been altered by SFA and MUFA diets.

NKT cells also recognize α-galactosylceramide (α-GalCer), a marine sponge–derived glycolipid presented by CD1d (32). In the liver, hepatocytes are the main source of CD1d expression and antigen presentation to NKT cells (33). A previous study showed that endoplasmic reticulum stress and lipid accumulation in hepatocytes of leptin-deficient *ob/ob* mice decrease hepatocyte CD1d expression and NKT cell activation (22). In the current study, we demonstrate that dietary fatty acids cause a slight, but not statistically significant, decrease of CD1d expression in hepatocytes. More importantly, both dietary fatty acids in vivo and fatty acid treatment in vitro significantly reduced the ability of hepatocytes to present endogenous antigen to NKT cells. The ability of hepatocytes to present an exogenous antigen (α-GalCer) was not affected by either high fatty acid diet treatment in vivo or fatty acid treatment in vitro. This leads us to conclude that high dietary fatty acids either reduce the endogenous antigen for NKT cells or interfere with the interaction between the endogenous antigen and CD1d. However, VanderLaan et al. have shown that dendritic cells preincubated with serum from high-fat diet–fed mice can stimulate NKT cells (34). This difference could be due to the difference between hepatocytes and dendritic cells in response to high-fat diets. In fact, our study also found a significant difference between hepatocytes and Kupffer cells in their ability of presenting endogenous antigen to NKT cells (see supplementary data), but Kupffer cells are equally effective in presenting exogenous antigen (α-GalCer) to NKT cells. Whether dietary fatty acids enhance or reduce the ability of Kupffer cells to present endogenous antigen is worth future investigation.

Previous studies have also shown that CD1d-mediated antigen presentation is regulated by both mitogen-activated protein kinases (MAPK) and protein kinase C (PKC) δ activities (35, 36). Inhibition of PKCδ significantly impairs antigen presentation by CD1d to NKT cells (35). In addition, it is known that dietary fatty acids regulate PKC activity (37). Compared with PUFA (DHA), SFA (PA) results in much less PKC activity in human hepatoma cells. It is possible that the change of dietary fatty acid composition alters PKC activity and impairs antigen presentation by CD1d to NKT cells. We are currently examining this hypothesis.

CONCLUSION

We have shown that high dietary SFA and MUFA, but not PUFA, cause hepatic NKT cell depletion, thus contributing to the formation of insulin resistance and hepatic steatosis. Dietary fatty acid–induced hepatic NKT cell depletion is

most likely the result of impaired CD1d-dependent endogenous antigen presentation by hepatocytes to NKT cells. Further studies to identify the endogenous antigen that responds to dietary fatty acid alteration will help to elucidate the pathogenesis of diet-induced obesity, insulin resistance, and NAFLD, and may have profound implications in identifying targets for therapy in obesity-related diseases.

The authors thank the Hopkins Digestive Diseases Basic Research Development Center for providing technical support and the National Institutes of Health Tetramer Core Facility for providing CD1d tetramer.

REFERENCES

1. Hla, T. 2005. Immunology: enhanced: dietary factors and immunological consequences. *Science.* **309:** 1682–1683.
2. Costabile, M., C. S. Hii, M. Melino, C. Easton, and A. Ferrante. 2005. The immunomodulatory effects of novel beta-oxa, beta-thia, and gamma-thia polyunsaturated fatty acids on human T lymphocyte proliferation, cytokine production, and activation of protein kinase C and MAPKs. *J. Immunol.* **174:** 233–243.
3. Finstad, H. S., H. Dyrendal, M. C. Myhrstad, H. Heimli, and C. A. Drevon. 2000. Uptake and activation of eicosapentaenoic acid are related to accumulation of triacylglycerol in Ramos cells dying from apoptosis. *J. Lipid Res.* **41:** 554–563.
4. Smith, A. G., P. A. Sheridan, R. J. Tseng, J. F. Sheridan, and M. A. Beck. 2009. Selective impairment in dendritic cell function and altered antigen-specific CD8+ T-cell responses in diet-induced obese mice infected with influenza virus. *Immunology.* **126:** 268–279.
5. Shaikh, S. R., D. Mitchell, E. Carroll, M. Li, J. Schneck, and M. Edidin. 2008. Differential effects of a saturated and a monounsaturated fatty acid on MHC class I antigen presentation. *Scand. J. Immunol.* **68:** 30–42.
6. Li, Z., M. J. Soloski, and A. M. Diehl. 2005. Dietary factors alter hepatic innate immune system in mice with nonalcoholic fatty liver disease. *Hepatology.* **42:** 880–885.
7. Harte, R. A., E. A. Kirk, M. E. Rosenfeld, and R. C. LeBoeuf. 1999. Initiation of hyperinsulinemia and hyperleptinemia is diet dependant in C57BL/6 mice. *Horm. Metab. Res.* **31:** 570–575.
8. Vessby, B., M. Unsitupa, K. Hermansen, G. Riccardi, A. A. Rivellese, L. C. Tapsell, C. Nalsen, L. Berglund, A. Louheranta, B. M. Rasmussen, et al. 2001. Substituting dietary saturated for monounsaturated fat impairs insulin sensitivity in healthy men and women: the KANWU Study. *Diabetologia.* **44:** 312–319.
9. Yuan, M., N. Konstantopoulos, J. Lee, L. Hansen, Z-W. Li, M. Karin, and S. E. Shoelson. 2001. Reversal of obesity- and diet-induced insulin resistance with salicylates or targeted disruption of IKKbeta. *Science.* **293:** 1673–1677.
10. Schenk, S., M. Saberi, and J. M. Olefsky. 2008. Insulin sensitivity: modulation by nutrients and inflammation. *J. Clin. Invest.* **118:** 2992–3002.
11. Pickup, J. C., and M. A. Crook. 1998. Is type II diabetes mellitus a disease of the innate immune system? *Diabetologia.* **41:** 1241–1248.
12. Grimble, R. F. 2002. Inflammatory status and insulin resistance. *Curr. Opin. Clin. Nutr. Metab. Care.* **5:** 551–559.
13. Ma, X., J. Hua, and Z. Li. 2008. Probiotics improve high fat diet-induced hepatic steatosis and insulin resistance by increasing hepatic NKT cells. *J. Hepatol.* **49:** 821–830.
14. Seki, S., Y. Habu, T. Kawamura, K. Takeda, H. Dobashi, T. Ohkawa, and H. Hiraide. 2000. The liver as a crucial organ in the first line of host defense: the roles of Kupffer cells, natural killer (NK) cells and NK1.1 Ag+ T cells in T helper 1 immune responses. *Immunol. Rev.* **174:** 35–46.
15. Exley, M. A., and M. J. Koziel. 2004. To be or not to be NKT: natural killer T cells in the liver. *Hepatology.* **40:** 1033–1040.
16. Schumann, J., F. Facciotti, L. Panza, M. Michieletti, F. Compostella, A. Collmann, L. Mori, and G. De Libero. 2007. Differential alteration of lipid antigen presentation to NKT cells due to imbalances in lipid metabolism. *Eur. J. Immunol.* **37:** 1431–1441.
17. Potter, J. J., V. W. Yang, and E. Mezey. 1989. Influence of growth hormone on the synthesis of rat liver alcohol dehydrogenase in primary hepatocyte culture. *Arch. Biochem. Biophys.* **274:** 548–555.
18. Webb, T. J., R. A. Litavecz, M. A. Khan, W. Du, J. Gervay-Hague, G. J. Renukaradhya, and R. R. Brutkiewicz. 2006. Inhibition of CD1d1-mediated antigen presentation by the vaccinia virus B1R and H5R molecules. *Eur. J. Immunol.* **36:** 2595–2600.
19. Webb, T. J., R. L. Giuntoli II, O. Rogers, J. Schneck, and M. Oelke. 2008. Ascites specific inhibition of CD1d-mediated activation of natural killer T cells. *Clin. Cancer Res.* **14:** 7652–7658.
20. Musso, G., R. Gambino, F. De Michieli, M. Cassader, M. Rizzetto, M. Durazzo, E. Faga, B. Silli, and G. Pagano. 2003. Dietary habits and their relations to insulin resistance and postprandial lipemia in nonalcoholic steatohepatitis. *Hepatology.* **37:** 909–916.
21. Cortez-Pinto, H., L. Jesus, H. Barros, C. Lopes, M. C. Moura, and M. E. Camilo. 2006. How different is the dietary pattern in non-alcoholic steatohepatitis patients? *Clin. Nutr.* **25:** 816–823.
22. Yang, L., R. Jhaveri, J. Huang, Y. Qi, and A. M. Diehl. 2007. Endoplasmic reticulum stress, hepatocyte CD1d and NKT cell abnormalities in murine fatty livers. *Lab. Invest.* **87:** 927–937.
23. Donnelly, J. E., D. K. Sullivan, B. K. Smith, D. J. Jacobsen, R. A. Washburn, S. L. Johnson, J. O. Hill, M. S. Mayo, K. R. Spaeth, and C. Gibson. 2008. Alteration of dietary fat intake to prevent weight gain: Jayhawk Observed Eating Trial. *Obesity (Silver Spring).* **16:** 107–112.
24. Oakes, N. D., G. J. Cooney, S. Camilleri, D. J. Chisholm, and E. W. Kraegen. 1997. Mechanisms of liver and muscle insulin resistance induced by chronic high-fat feeding. *Diabetes.* **46:** 1768–1774.
25. Seino, K., and M. Taniguchi. 2005. Functionally distinct NKT cell subsets and subtypes. *J. Exp. Med.* **202:** 1623–1626.
26. Meade, C. J., and J. Mertin. 1978. Fatty acids and immunity. *Adv. Lipid Res.* **16:** 127–165.
27. Lantz, O., and A. Bendelac. 1994. An invariant T cell receptor alpha chain is used by a unique subset of major histocompatibility complex class I-specific CD4+ and CD4–8- T cells in mice and humans. *J. Exp. Med.* **180:** 1097–1106.
28. Mlekusch, W., C. Celedin, R. C. Aloia, and R. Moller. 1993. Effect of a high fat diet on phospholipid class distribution and fatty acid composition in rat liver. *Int. J. Biochem.* **25:** 1539–1547.
29. Porubsky, S., A. O. Speak, B. Luckow, V. Cerundolo, F. M. Platt, and H. J. Grone. 2007. Normal development and function of invariant natural killer T cells in mice with isoglobotrihexosylceramide (iGb3) deficiency. *Proc. Natl. Acad. Sci. USA.* **104:** 5977–5982.
30. Zhou, D., J. Mattner, C. Cantu III, N. Schrantz, N. Yin, Y. Gao, Y. Sagiv, K. Hudspeth, Y-P. Wu, T. Yamashita, et al. 2004. Lysosomal glycosphingolipid recognition by NKT cells. *Science.* **306:** 1786–1789.
31. Ichi, I., K. Nakahara, K. Kiso, and S. Kojo. 2007. Effect of dietary cholesterol and high fat on ceramide concentration in rat tissues. *Nutrition.* **23:** 570–574.
32. Kawano, T., J. Cui, Y. Koezuka, I. Toura, Y. Kaneko, K. Motoki, H. Ueno, R. Nakagawa, H. Sato, E. Kondo, et al. 1997. CD1d-restricted and TCR-mediated activation of valpha14 NKT cells by glycosylceramides. *Science.* **278:** 1626–1629.
33. Mandal, M., X. R. Chen, M. L. Alegre, N. M. Chiu, Y. H. Chen, A. R. Castano, and C. R. Wang. 1998. Tissue distribution, regulation and intracellular localization of murine CD1 molecules. *Mol. Immunol.* **35:** 525–536.
34. VanderLaan, P. A., C. A. Reardon, Y. Sagiv, L. Blachowicz, J. Lukens, M. Nissenbaum, C. R. Wang, and G. S. Getz. 2007. Characterization of the natural killer T-cell response in an adoptive transfer model of atherosclerosis. *Am. J. Pathol.* **170:** 1100–1107.
35. Brutkiewicz, R. R., C. A. Willard, K. K. Gillett-Heacock, M. R. Pawlak, J. C. Bailey, M. A. Khan, M. Nagala, W. Du, J. Gervay-Hague, and G. J. Renukaradhya. 2007. Protein kinase C delta is a critical regulator of CD1d-mediated antigen presentation. *Eur. J. Immunol.* **37:** 2390–2395.
36. Renukaradhya, G. J., T. J. R. Webb, M. A. Khan, Y. L. Lin, W. Du, J. Gervay-Hague, and R. R. Brutkiewicz. 2005. Virus-induced inhibition of CD1d1-mediated antigen presentation: reciprocal regulation by p38 and ERK. *J. Immunol.* **175:** 4301–4308.
37. Sung, M., I. Kim, M. Park, Y. Whang, and M. Lee. 2004. Differential effects of dietary fatty acids on the regulation of CYP2E1 and protein kinase C in human hepatoma HepG2 cells. *J. Med. Food.* **7:** 197–203.