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Accelerated onset of diabetes in NOD mice fed a refined high-fat diet

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Abstract

Objective: Type 1 diabetes results from autoimmune events influenced by environmental variables, including changes in diet. This study investigated how feeding refined versus unrefined (aka ‘chow’) diets affects the onset and progression of hyperglycemia in non-obese diabetic (NOD) mice.

Methods: Female NOD mice were fed either unrefined diets or matched refined low- and high-fat diets. The onset of hyperglycemia, glucose tolerance, food intake, energy expenditure, circulating insulin, liver gene expression, and microbiome changes were measured for each dietary group.

Results: NOD mice consuming unrefined (chow) diets developed hyperglycemia at similar frequencies. By contrast, mice consuming the defined high-fat diet had an accelerated onset of hyperglycemia compared to the matched low-fat diet. There was no change in food intake, energy expenditure, or physical activity within each respective dietary group. Microbiome changes were driven by diet type, with chow diets clustering similarly while refined low- and high-fat bacterial diversity also grouped closely. In the defined dietary cohort, liver gene expression changes in high-fat-fed mice were consistent with a greater frequency of hyperglycemia and impaired glucose tolerance.

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Conclusion: Glucose intolerance is associated with enhanced frequency of hyperglycemia in female NOD mice fed a defined high-fat diet. Using an appropriate matched control diet is an essential experimental variable when studying changes in microbiome composition and diet as a modifier of disease risk.

Keywords

autoimmunity; diabetes; diet; non-obese diabetic (NOD) mouse model; microbiome; obesity

1. Introduction

Type 1 diabetes (T1D) is an autoimmune disease associated with immune cell infiltration into pancreatic tissue, targeting of pancreatic islet β-cells, and subsequent reductions in circulating insulin [1–3]. There are multiple proposed risk factors for T1D, including genetic components, such as the inheritance of specific MHC and HLA alleles [4]. Indeed, the MHC and HLA alleles in mice and humans show similar sequence specificity [5]. In addition, other putative environmental modifiers of disease risk, coupled with genetic susceptibility, are proposed to increase the likelihood of developing T1D. For example, viral exposure [6], lack of sunlight [7,8], hygiene [9], and diet [10] have all been considered disease-modifying risk factors.

Diet is critical because greater access to calorically dense foods and a sedentary lifestyle in modern society promotes obesity which is associated with insulin resistance and increased risk for diabetes [11]. Individuals with genetic risk for T1D are subject to the same lifestyle and environmental factors as those with the propensity to develop T2D (Type 2 diabetes). Indeed, insulin resistance has been proposed as a possible explanation for the rise in T1D [12]. However, appropriate modeling of dietary factors in pre-clinical models has been hampered by inappropriate or suboptimal experimental designs, concerns which have been reviewed previously [13–16].

One additional factor proposed to contribute to T1D, and potentially influenced by diet, is alterations to the gut microbiome. Indeed, studies have been conducted to examine the relationship between microbiome changes and diabetes onset [17]. For example, germ free mice develop diabetes at the same rate as mice housed in specific pathogen free conditions [18,19]. However, the presence or absence of gut microorganisms undoubtedly influence diabetes within specific genetic contexts [19,20]. Thus, whether changes in glycemia alter the gut microbe composition or changes in bacterial diversity in the host digestive system influence hyperglycemia are not fully resolved.

In the present study, we demonstrate that comparison of appropriate control diets is critical for interpreting hyperglycemia onset in NOD mice consuming a high-fat diet. When comparing NOD mice consuming refined matched low- and high-fat diets, there is a greater incidence of hyperglycemia in mice consuming the high-fat diet. When mice consume an unrefined (aka ‘chow’) diet, doubling the fat content of the chow diet did not influence the onset of hyperglycemia. These changes also appear to be mirrored in microbiome composition and diversity which were significantly impacted by the type of diet (unrefined

versus refined) but differed less when compared within each respective matched dietary group.

2. Methods

2.1 Animals and body composition measurements.

2.1.1 Unrefined Diet (aka chow) Studies—Fifty-six NOD/ShiLtJ (Stock # 001976) female mice were purchased from the Jackson Laboratory (Bar Harbor, ME) at 6 weeks of age. Mice were allowed to acclimate to a 12 hr light-dark cycle at 24°C in the animal facility for a minimum of one week prior to experimental procedures. All mice were given Lab Diet 5001 upon arrival at the facility. Forty of the mice were randomized into groups of twenty mice receiving either Lab Diet 5001 (13% kcal from fat; Lab Diet, St. Louis, MO) or Lab Diet 5015 (26% kcal from fat; Lab Diet, St. Louis, MO) with twenty mice beginning on Lab Diet 5015 at 8 weeks of age. Randomization was conducted using baseline body mass to confirm that all groups began with body mass values that were not statistically different. The animals monitored for diabetes onset were multi-housed and had *ad libitum* access to water and food. Body mass and body composition measurements (fat, lean mass, and fluid mass) were started at 8 or 10 weeks of age and then every two weeks until onset of hyperglycemia using a Bruker Minispec LF110 Time-Domain NMR system. Blood glucose was measured from tail blood using a Bayer Contour Glucometer at baseline (start of study) and twice per week thereafter. Animals were euthanized at onset of hyperglycemia (2 consecutive daily measurements ≥250 mg/dL) or by 20 weeks of age. Frequency of diabetes in female NOD mice in our facility is 75% by 30 weeks of age. Animals were fasted for 4 h followed by CO_2 asphyxiation and cervical dislocation. Pancreata were fixed in 10% (volume/volume) neutral buffered formalin for histological analysis. Trunk blood was collected and the serum fraction was separated for downstream analysis. The additional sixteen mice were randomized using the same strategy and used for metabolic cage studies as described below.

2.1.2 Refined Diet Studies—A separate cohort of fifty-six NOD/ShiLtJ (Stock # 001976) female mice were purchased from the Jackson Laboratory (Bar Harbor, ME) at 6 weeks of age. Mice were allowed to acclimate to a 12 hr light-dark cycle at 24°C in the animal facility for a minimum of one week prior to experimental procedures. For studies monitoring hyperglycemia, the animals were multi-housed and had *ad libitum* access to water and 10% kcal purified low-fat diet (LF) (catalog #: D12450H; Research Diets, Inc, New Brunswick, NJ) upon arrival at the facility. Body mass and body composition measurements (fat, lean mass, and fluid mass) were generated starting at 9 weeks of age and then weekly until onset of hyperglycemia using a Bruker Minispec LF110 Time-Domain NMR system. Blood glucose was measured from tail blood using a Bayer Contour Glucometer at baseline (start of study) and after randomization (as described in 2.1.1) and assignment to dietary group, twice per week thereafter. At 8 weeks of age, the mice were given either LF (n=20) or 45% kcal high fat diet (HF) (n=20; catalog#: D12451; Research Diets, Inc, New Brunswick, NJ). Animals were euthanized at onset of hyperglycemia (2 consecutive daily measurements ≥250 mg/dL) or by 30 weeks of age. Animals were fasted for 4 h followed by CO_2 asphyxiation and cervical dislocation. Liver was snap frozen in

liquid nitrogen and pancreata were fixed in 10% (volume/volume) neutral buffered formalin for histological analysis. Trunk blood was collected, and the serum fraction was separated for downstream analysis. The additional sixteen mice were randomized using the same strategy and used for metabolic cage studies as described below. All animal procedures were approved by Institutional Animal Care and Use Committees at Pennington Biomedical Research Center and the University of Tennessee.

2.2 Glucose Tolerance Tests.

Unrefined (aka chow) Diet and Refined Diet—A glucose tolerance test (GTT) was performed in 14 week old female NOD mice following a 4 h fast using intraperitoneal (i.p.) injections of glucose at 2.5g/kg body weight. For both cohorts receiving GTTs, blood glucose was measured from tail blood using the Bayer Contour Glucometer.

2.3 Metabolic Cage Analyses

For metabolic cage measurements, eight mice on each of the four diets as described above were placed at eleven weeks of age into training cages for one week of acclimation. Thus, they were twelve weeks of age upon entry into the metabolic cage (Promethion Metabolic Screening Cages, Sable Systems International, Las Vegas, NV) for continuous measurements. Corn cob bedding was included and there was an intake manifold (a small metal tube that runs along the perimeter of the cage to pull air). The training cage was exactly the same (bedding, dimensions, etc.) as the testing cage, minus the manifold on the perimeter of the cage. Mice were single housed in the training cages and also in the metabolic cages during measurements.

2.4 Serum Hormone and Gene Expression Measurements

Serum insulin was measured using the Mouse Insulin ELISA kit from Mercodia (Uppsala, Sweden) according to the manufacturer's instructions. RNA was isolated from liver using RNeasy extraction kits (Qiagen) and cDNA synthesis was carried out using iScript (Bio-Rad). Real-time PCR was conducted using SYBR Green (Bio-Rad) run on a CFX Opus instrument (Bio-Rad) with gene specific primers (available upon request). Gene expression was normalized to the Ribosomal Protein S9 (Rs9) gene using the delta-delta Ct method.

2.5 Stool Microbiome

Fresh stool samples were collected from each mouse in all groups, unrefined (13%), unrefined (26%), refined (10%), and refined (45%) fed mice, at 16 weeks of age into a 2.0 mL microcentrifuge tube and placed on ice prior to DNA isolation. DNA was prepared from fecal samples via bead beating and subsequent isolation using the QIAamp DNA Stool Mini Kit (Qiagen). The V4 region of the 16S rRNA gene was amplified via PCR using barcoded primer sequences. Amplicons were sequenced using the Illumina MiSeq platform (250-bp/paired-end reads). To reduce technical confounding from batch effects, all samples were sequenced at the same time and the sequencing order was randomized.

2.6 Microbiome Data Analysis

Amplicons were processed via Mothur v. 1.48.0 using default analysis settings [21]. Operational taxonomic units (OTUs) delimited at 97% identity were identified and classified according to SILVA reference files release 132 [22]. After application of standard quality control measures, the library size per sample ranged from 14827 to 1077789. A total of 2123 unique OTUs were observed demonstrating high-quality clustering. The mean sequencing error rate was very close to 0% based on parallel sequencing of a mock community. Estimates of alpha and beta diversities (intra- and inter-sample diversities, respectively) were obtained with Marker Data Profiling module of MicrobiomAnalyst 2.0 [23]. The alpha diversity was primarily estimated based on the observed OTU richness. Weighted and unweighted UniFrac distances were used to estimate beta diversity. Diversity metrics were calculated based on sequence counts scaled with cumulative sum scaling normalization.

2.7 Pancreas Immunohistochemistry

Embedding, sectioning, and staining of formalin-fixed paraffin-embedded (FFPE) tissues was conducted as described previously [24,25]. Five micron sections of FFPE tissue was cut onto slides for immunofluorescence staining assays and incubated overnight at room temperature with rat anti-Foxp3 (1:100, Invitrogen 14–5773-82). After three washes at five minutes each with TBST, slides were incubated with Vector Goat anti-Rat HRP polymer for 30 min then washed with TBST, followed by exposure to Biotium CF488 Tyramide (1 uM) in Biotium Amplification Buffer Plus for 10 minutes. Additional primary antibodies were Rabbit anti-CD3 (1:500; ab16669) used at room temperature for 1.5 hours and Rabbit anti-Iba1 (1:1000; Wako 019–19741) and Guinea Pig anti-Insulin (1:500; ab7842) used overnight at four degrees. After counterstain with Hoechst, the slides were mounted in Vectashield Vibrance. Insulitis scoring was conducted as previously described [24]. We used the following classification scheme to allow for a semiquantitative analysis: 0 indicates no visible infiltration; 1, visible peri-insulitis with <10% of islet occluded; 2, visible peri-insulitis with partially or complete immune cell encircling the islet but affecting less than half of the islet area; 3, invasive insulitis, defined as comprising 50% or more of the islet area being occluded with leukocytic infiltration. The number of islets quantified for each dietary group are represented as follows: total number of islets/total number of sections analyzed where each section is from an individual mouse. 13% unrefined (73/8), 26% unrefined (102/8), 10% refined (175/13), and 45% refined (182/11).

2.8 Statistical Analysis.

Statistical analysis was performed using GraphPad Prism 10.1.2 (GraphPad Software, La Jolla, CA). Outliers were detected using the ROUT method with the standard settings (Q = 1%). Otherwise, all data were analyzed by two-tailed Student’s t-test, one-way analysis of variance (ANOVA) using a Tukey’s post hoc, Chi Square analysis (Kaplan-Meier curves), Kruskal-Wallis (microbiome distribution) or repeated-measures ANOVA (for longitudinal measures of body weight and body composition). Data are represented as means ± SEM.

3. Results

3.1 High-fat fed NOD mice show a greater incidence of diabetes when compared with matched refined low-fat diet but display no differences in diabetes onset when consuming unrefined (aka chow) diets.

Female NOD mice were started on either unrefined (13% or 26% fat kcal) or refined (10% or 45% fat kcal) diets. A glucose tolerance test reveals a trend towards slower clearance of glucose in the unrefined (26%) relative to the unrefined (13%; Figure 1A). When NOD mice are fed refined diets, there is reduced glucose tolerance in the high-fat (45%) relative to the low-fat refined (10%) group (Figure 1B). When all mice were monitored for diabetes onset, those consuming the unrefined diets developed diabetes at similar frequencies (Figure 1C; compare black and red lines; $p = 0.20$, designated as n.s.). However, mice on refined diets reveal that high-fat feeding accelerated diabetes onset at a greater incidence when compared with matched low-fat controls (Figure 1C; compare green and blue lines; $p = 0.07$, designated by #). Thus, type of diet (unrefined versus refined) influences the time to hyperglycemia onset in female NOD mice.

3.2 Respiratory quotient (RQ) is influenced by diet.

Body mass was similar between each group on unrefined (Supplementary Figure 1A) and refined diets (Supplementary Figure 1B). Fat mass was also comparable between mice within each respective diet group (Supplementary Figure 1C and Figure 1D). There was no difference in lean mass in each dietary group diet (compare Supplementary Figures 1E with 1F).

Mice on both unrefined (Supplementary Figure 2A) and refined (Supplementary Figure 2B) diets displayed similar energy expenditure across one week of measurements. When examining spontaneous physical activity, mice on unrefined diets (Supplementary Figure 2C) were not different. In addition, there were no changes in food intake (Supplementary Figure 2D) or water intake (Supplementary Figure 2E) in mice consuming the unrefined diets. Furthermore, mice fed refined (10% and 45%) also had no differences in physical activity (Supplementary Figure 2F), food intake (Supplementary Figure 2G), or water intake (Supplementary Figure 2H).

When fed unrefined diets, mice on the higher fat version (26% kcal) had greater RQ values across the week which cycled by light/dark cycle (Figure 2A). The cumulative RQ was higher in the 26% fat group versus the 13% fat group (Figure 2B), which was consistent with greater RQ in both the light and dark cycles in mice fed these unrefined diets (Figure 2C). Alternatively, RQ was greater in mice given the low-fat (10% kcal) compared with the high-fat (45% kcal) refined diet (Figure 2D). These data were consistent when observed cumulatively (Figure 2E) and when parsed out by light and dark cycles (Figure 2F).

3.3 High-fat feeding promoted elevated circulating insulin in normoglycemic mice but produced no obvious alterations in immune cell invasion into pancreatic islets.

Serum insulin is influenced by fat content in the diet with mice eating the higher fat unrefined diet displaying greater insulin quantities prior to disease onset (Figure 3A; NG,

normoglycemic). Once mice became hyperglycemic, serum insulin levels were detectably reduced in mice consuming the unrefined diet with higher fat content (Figure 3A; HG, hyperglycemic). Similar results were obtained with mice consuming the refined low- and high-fat diets (Figure 3B). Within the refined diets, the greater incidence of hyperglycemia occurred in the high fat group (Figure 1); therefore, we next measured immune cell infiltration in and near islets as a possible explanation for these findings. Insulitis scoring revealed similar immune cell infiltration patterns in both low-fat and high-fat fed mice (Figure 3C). Staining for specific immune cell types, such as regulatory T-cells (FoxP3+), T-lymphocytes (CD3+), and macrophages (IBA1+) were also congruent with insulitis scoring patterns for each diet (Figure 3D). We noted that hyperglycemic mice had more severe insulitis scores when compared with normoglycemic mice. Finally, we observed that ICAM-1, a protein involved in cell-cell contacts and immune cell activation [2,26], did not appear to be altered by high-fat feeding (Supplementary Figure 3).

3.4 High-fat feeding alters liver gene expression patterns in female NOD mice.

NOD mice fed the refined high-fat diet show reduced hepatic expression of the *Acaca* and *Fasn* genes and increased expression of *Dgat1* and *Dgat2* (Figures 4A–D). This gene expression pattern is consistent with reduced hepatic *de novo* lipogenesis, while maintaining triglyceride storage capability, phenotypes documented in other strains of mice fed matched low- and high-fat diets [27]. Moreover, there was increased expression of *Slc2a2* and *Pck1*, which encode GLUT2 and PEPCK, respectively, in NOD mice fed a refined high-fat diet (Figures 4E & F). These latter changes in gene expression could support increased hepatic glucose production as one possible mechanism explaining an increased onset of hyperglycemia in mice fed a refined high-fat diet. Consistent with similar onset of diabetes in the unrefined (chow) diets (Figure 1), there were no major gene expression changes between the unrefined diets in the livers of these mice (data not shown).

3.5 Alpha and beta diversity are highly influenced by unrefined versus refined diets.

With no major changes in body mass, body composition, or energy expenditure between the mice on each respective type of diet (Supplementary Figures 1 and 2), we next tested the premise that diet influences microbiome changes. After filtering out low expression and low variance operational taxonomic units (OTUs), the remaining OTUs represented 38 unique bacterial genera across five phyla (Actinobacterial, Bacteroidetes, Firmicutes, Tenericutes, and Verrucomicrobia; Supplementary Figure 4A). At the genus level, organismal diversity clustered by type of diet (13% unrefined similar to 26% unrefined; 10% refined similar to 45% refined; Supplementary Figure 4B).

Within the unrefined diet groups, a much higher compositional diversity (alpha diversity, measured by Shannon diversity index) was observed compared to the stool samples from the refined diets (Fig. 5A), resulting in a highly statistically significant difference across the four dietary groups (Kruskall-Willis H=25.3, p-value = 1.31E-05; Fig 5A). This significance was primarily driven by the diversity indices of the unrefined groups compared to the indices for the refined diet groups. The alpha-diversity indices were more similar between unrefined groups (within group comparison) than to refined 10% and 45% fat diets (across group comparison). These results argue for a qualitative difference in the richness of taxa

in the unrefined-fed animals compared to the mice fed the refined 10% and 45% fat diets. The beta-diversity index further estimated the differences in microbiota composition across the four groups. As seen in the principal component analysis plot (Fig. 5B), samples belonging to the unrefined groups clustered together, whereas a separate cluster was observed for the refined diet samples. Testing via the PERMANOVA method showed an overall statistically significant difference across the four groups ($F=21.2$, p-value <0.001). Post-hoc testing showed the greatest pairwise differences between unrefined 13% and refined 45% diet ($F=40.03$, $p<0.001$) and unrefined 26% versus refined 10% diet ($F=22.1$, $p<0.001$). Comparing defined LF- to HF-diet driven microbiota communities directly, we observed 52 OTUs that were deemed statistically significantly different (Supplementary Table 1). Community members with the highest representation in this group include members of the genera Staphylococcus, Clostridium senso stricto, and Turicibacter; all these are significantly reduced in the HF diet-shaped communities. Conversely, members of the phylum proteobacteria – belonging to the family Desulfovibrionaceae – show increased representation in the HF-shaped community. While Desulfovibrio sp. have been linked to metabolic syndrome inflammation [28], direct links to autoimmunity are unclear at this time. In summary, we conclude that microbiome changes are influenced by type of diet (i.e., unrefined versus refined) but do not necessarily contribute to increased or decreased outcome measures used in this study.

4. Discussion

The prevalence of both major forms of diabetes, T1D and T2D, has increased over the past two decades [29]. While many possibilities may exist to explain these increases in disease prevalence, diet is certainly one likely risk factor. Here, we have used the NOD mouse, the gold standard preclinical model for T1D [30], to test the hypothesis that high-fat feeding alters the course of hyperglycemia onset. We found that feeding a refined high-fat diet increases the prevalence of diabetes in female NOD mice when compared with a matched refined low-fat diet. Therefore, our data does not support previous work showing that high-fat diet prevents autoimmune diabetes in NOD mice [31]. We suspect this difference in experimental outcomes is due to the distinct dietary conditions used in each study.

High-fat diets have been primarily used in mice not prone to autoimmune disease, focusing on the development of obesity and associated metabolic outcomes, such as impaired glucose tolerance and insulin resistance [32]. However, many such studies using high-fat diets have not selected an appropriate control diet in the experimental design, which drastically alters the interpretation of the data [14–16]. Herein, we used two different unrefined (aka chow) diets and two distinct, refined matched low- and high-fat diets to investigate their impact on hyperglycemia development in female NOD mice.

NOD mice eating the refined high-fat diet developed hyperglycemia more frequently than their counterparts on a matched low-fat diet (Figure 1). We found that NOD mice consuming unrefined ‘chow’ diets developed diabetes at similar rates but also exhibited hyperglycemia earlier than mice on refined diets (Figure 1). A previous study concluded that high-fat feeding prevents or slows the development of hyperglycemia in female NOD mice, but did so by comparing an unrefined (chow) diet to a refined high-fat diet [31].

When comparing the unrefined dietary groups with the refined HF group in the present study (Figure 1C – compare black line to blue line), our experiments recapitulate this prior report. However, when mice are fed appropriately matched refined low- and high-fat diets, we found that high-fat diet consumption promotes the development of hyperglycemia (Figure 1C – compare green line to blue line). Consequently, the general conclusion that high fat prevents or slows development of hyperglycemia may need revision, because with the correct matched control diet, we observed that a refined high-fat diet advanced the pathological condition. Thus, selection of the most appropriate control diet is a highly essential factor influencing the interpretation of the study results [14–16].

We suspect that the impaired glucose tolerance observed in high-fat fed mice (Figure 1) is an early predictor of hyperglycemia in mice as it is in humans [33,34]. It is possible that high-fat feeding imparts greater stress on β-cells, promotes hepatic glucose production, or both leading to impaired glucose tolerance. We note that mice consuming the refined high-fat diet had reduced expression of genes involved with *de novo* lipogenesis (DNL; Figures 4A and 4B) concomitant with greater expression of genes controlling triglyceride synthesis in the liver (Figures 4C and 4D). These gene expression results in NOD mice are consistent with the suppression of DNL in the BDF1 mouse model fed a high-fat diet [27] and in humans [35]. In addition, we found that refined high-fat fed mice had increased expression of the genes encoding phosphoenolpyruvate carboxykinase (*Pck1* encoding PEPCK) and (*Slc2a2* encoding GLUT2) in the liver (Figures 4E and 4F). Fatty acids increase expression of PEPCK in culture and enhanced hepatic PEPCK expression leads to glucose intolerance *in vivo* [36–38]. Collectively, these findings are congruent with refined high-fat feeding altering liver metabolism in a manner consistent with the acceleration of hyperglycemia in NOD mice (Figure 1). Finally, we also note the larger amount of sucrose in the refined diets (Figures 2D–F) was likely to be a major factor influencing RQ values. Sucrose is low to absent from traditional unrefined diets, including those used in the present study (Figures 2A–C). Sucrose and other carbohydrate sources are known to influence the RQ value [39–41].

Our preclinical data showing changes in glucose tolerance preceding hyperglycemia are compatible with alterations in glucose tolerance predicting the likelihood of T1D onset in humans [42]. Additionally, a high-fat diet could promote chronic low-grade inflammation that exacerbates the disease factors associated with autoimmunity. For example, inflammatory stimuli enhance MHC II complexes on antigen-presenting cells [43], which in T1D may promote greater disease risk. Furthermore, glucose intolerance, insulin resistance, and obesity intensify or accelerate the onset of many diseases and also make treating such conditions more difficult [44–46].

Another potential factor contributing to autoimmunity is the microbiome. Indeed, many studies report investigating the relationship between microbiome changes and diabetes onset [17]. For example, germ free mice develop diabetes at the same rate as mice housed in specific pathogen free conditions [18,19]. However, the presence or absence of gut microorganisms undoubtedly influence diabetes within specific genetic contexts [19,20]. When assessing alterations in the microbiome, it appears that phylogenetic diversity fluctuates with type of diet (e.g., unrefined versus refined), but may be modulated less drastically within matched dietary groups. Thus, microbiome changes are a readout of changes in food composition

(i.e., unrefined to refined; ref. [13] and present data). However, whether such alterations are directly associated with the onset or progression of autoimmune disease and to what extent microbiome changes modify disease risk is not entirely understood. Nevertheless, a consistent viewpoint is that comparison of an experimental diet to its appropriate matched control diet is critical for a rigorous interpretation of results [refs. [13–16] and present data].

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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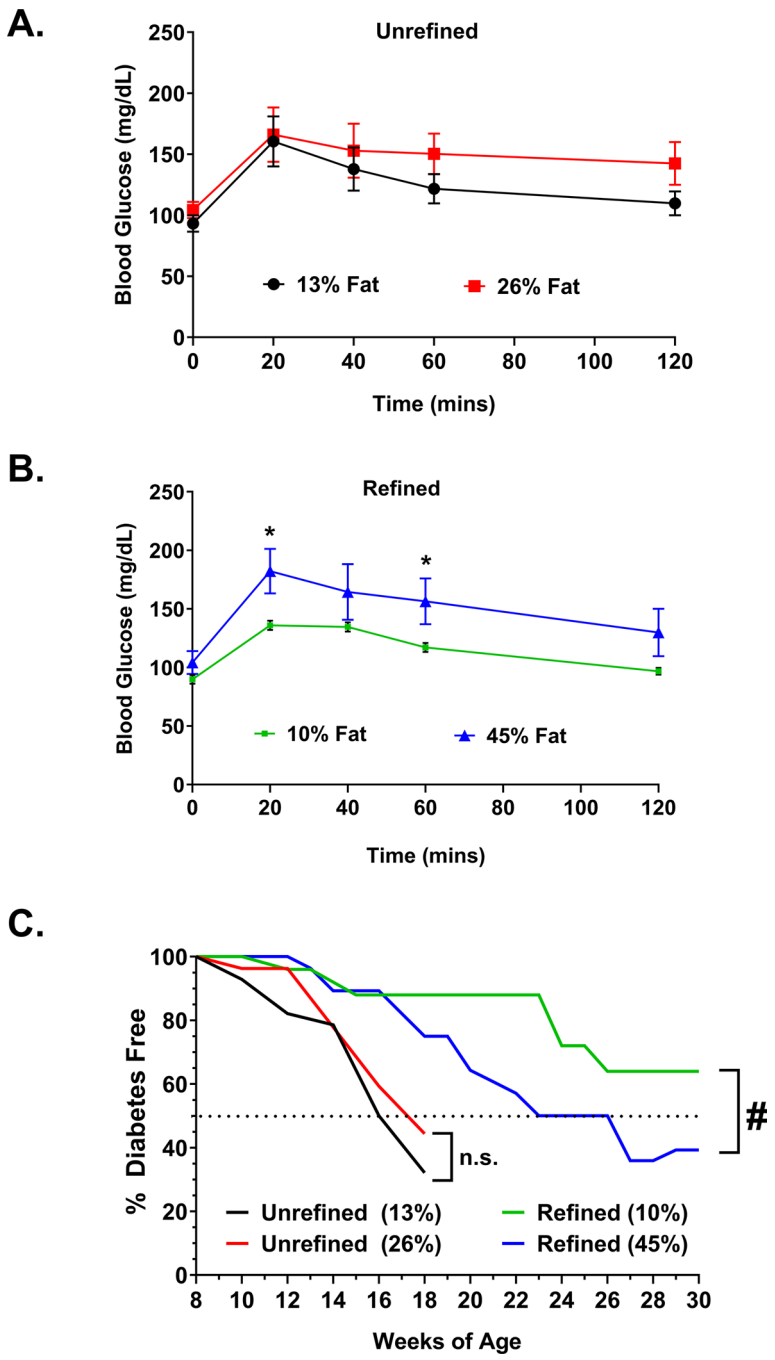

Figure 1. Refined high-fat diet promotes glucose intolerance and increased incidence of hyperglycemia in female NOD mice.

A. Glucose tolerance tests (GTTs) conducted in 14 week old (wo) female NOD mice. **B**. Glucose tolerance tests conducted in 14–16 wo female NOD mice. **C**. The incidence of diabetes was monitored twice weekly starting at 8 weeks of age (n = 25–28 per dietary group). Mice were considered diabetic after two consecutive values ≥ 250mg/dL. Dashed line indicates 50% threshold. GTT data are shown as means ± SEM while diabetes incidence was plotted using Kaplan-Meier curves. #, $p < 0.1$; *, $p < 0.05$; n.s., not significant.

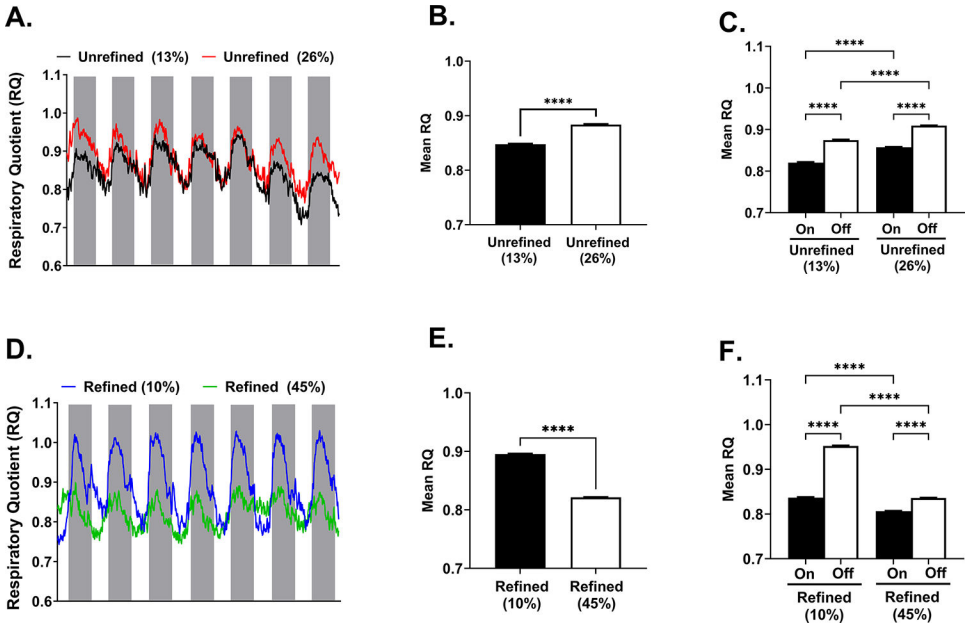

Figure 2. Respiratory quotient is impacted by diet in female NOD mice.

Respiratory quotient in mice fed either unrefined (**A – C**) or refined diets (**D – F**). **A, D**. RQ across the light (white sections) and dark (grey sections) cycle over a seven day period. **B, E**. Cumulative mean RQ over seven days. **C, F**. Cumulative mean RQ parsed into lights on and lights off over seven days in metabolic cages. Bar graphs represent data as means ± SEM. ****, $p < 0.0001$. n = 8 per group.

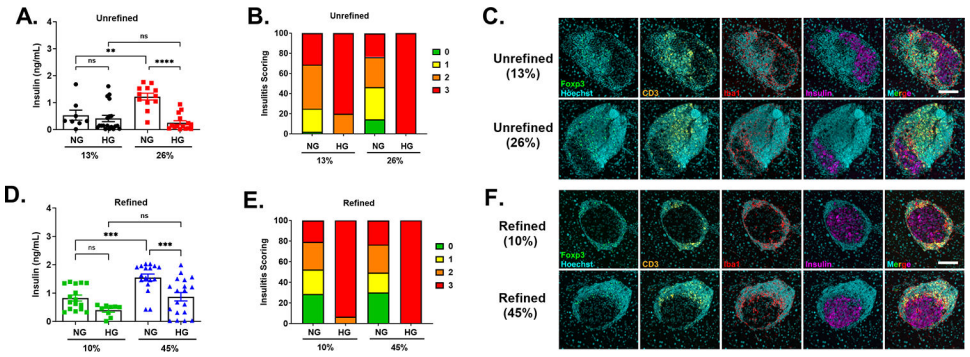

Figure 3. Circulating insulin is influenced by dietary fat content and reflective of diabetes status.

Serum insulin values from mice fed unrefined (**A;** n = 8–19) and refined (**D**; n = 9–19) diets per group separated by glucose threshold of < 250 mg/dL (normoglycemia; NG) and ≥ 250 mg/dL (hyperglycemia; HG). Scoring of immune cell infiltration (insulitis) using a 0–3 scale described in the methods separated by glucose threshold of < 250 mg/dL (NG) and ≥ 250 mg/dL (HG) from mice fed unrefined diets (**B;** n = 16) and refined (**E**; n = 24). **D**. Five color IF imaging showing single channel and merge of FFPE pancreatic tissue stained for individual immune cells (i.e. Foxp3, CD3, and Iba1) and insulin positive cells from mice fed unrefined (**C;** n = 6) and refined diets (**F**; n = 6). Islets of similar size were compared. Scale bar = 100 μm.

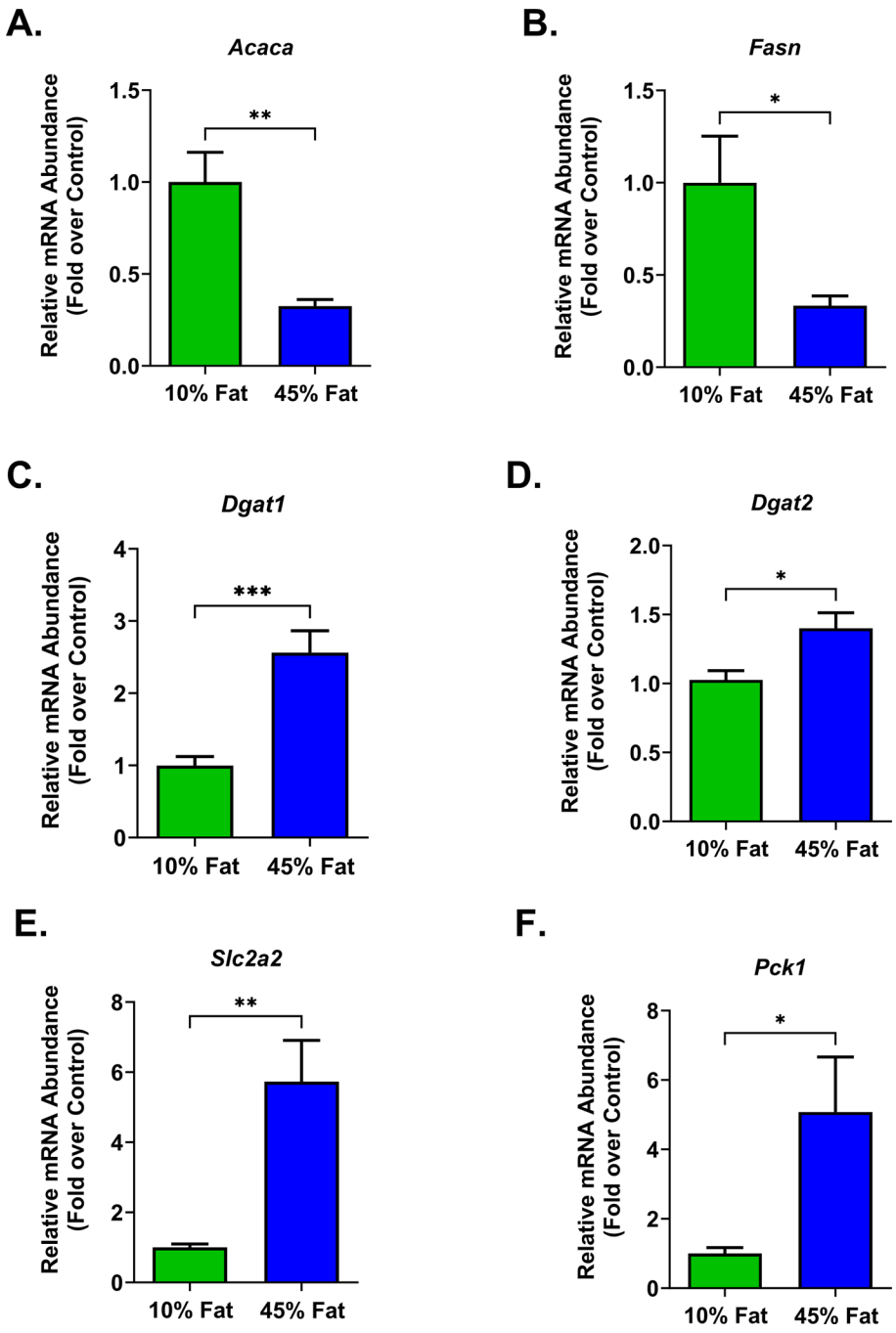

Figure 4. Liver gene expression is altered by refined high-fat feeding.

Expression of *Acaca* (**A**), *Fasn* (**B**), *Dgat1* (**C**), *Dgat2*, (**D**) *Slc2a2* (**E**), and *Pck1* (**F**) measured by RT-PCR using RNA isolated from liver (n = 8 per group). Data are presented as mean ± SEM, where * denotes $p < 0.05$, ** $p < 0$ .01 and *** $p < 0.001$.

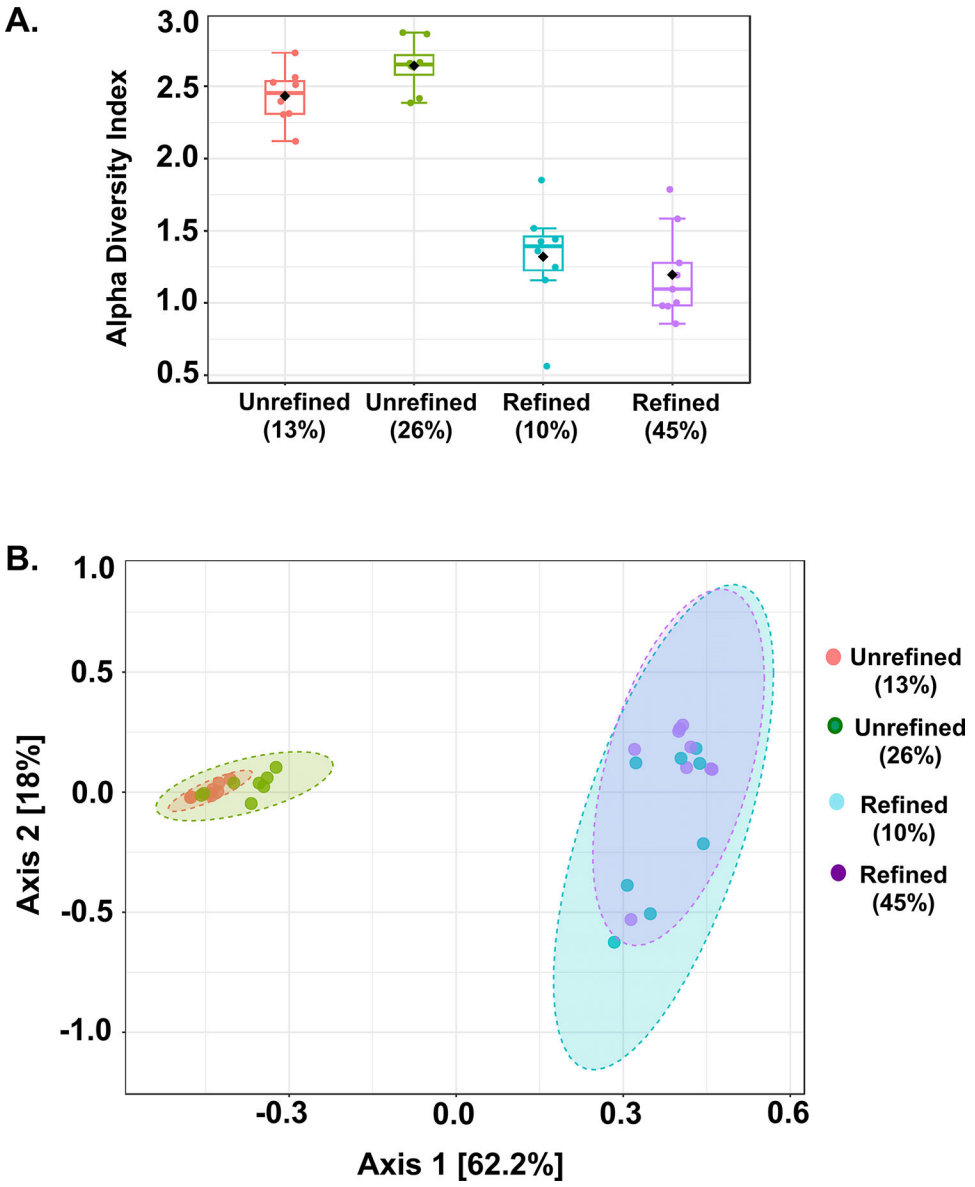

Figure 5. Alpha and beta diversity show clustering largely by dietary type (unrefined versus refined).

A. The observed compositional diversity is greater in the unrefined (chow) fed groups compared to the refined low- and high-fat fed mice. **B**. The differences in microbiota composition across the four distinct dietary groups. n = 8 per dietary group.