Metabolic signatures in nutrition and health : short-term diet response, sexual dimorphism and hormone chronobiology

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Chapter 3

Vegan and animal meal composition and timing influence glucose and lipid related postprandial metabolic profiles

Based on

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Submitted for Publication 2018

ABSTRACT

Flexitarian dieting is increasingly associated with health benefits. The study of postprandial metabolic response to vegan and animal diets is essential to decipher how specific diet components may mediate metabolic changes. Therefore, a randomized, cross-over, controlled vegan versus animal diet challenge was conducted on 21 healthy participants. Postprandial metabolic measurements were conducted at six timepoints during breakfast and lunch. Area under the curve analysis of the vegan diet response demonstrated higher glucose (EE 0.35), insulin (EE 0.38), triglycerides (EE 0.72) and 9 amino acids at breakfast (EE 4.72 to 209.32); and 6 lower health-promoting fatty acids at lunch (EE -0.1035 to -0.13) ($p < 0.05$). Glycemic and lipid parameters varied irrespective of diet type, demonstrating vegan and animal meals contained both health promoting and suboptimal nutrient combinations. The vegan breakfast produced the same elevated branched chain amino acid-associated metabolic signature as the animal diet from our fasting results, reflecting the low protein load in the animal and the higher branched chain amino acid load of the vegan breakfasts. Liberalization of the vegan menu to vegetarian and the animal menu to a Nordic-based diet could result in optimal metabolic signatures for both flexitarian diet strategies in future research.

INTRODUCTION

Vegan, vegetarian and animal-based omnivorous diets are naturally health promoting if they are well-balanced with sufficient macro and micronutrients to meet dietary requirements [1-6]. Mediterranean, Nordic and flexitarian (semi-vegetarian) diets are omnivorous diets with an emphasis on plant-based foods and have been shown to correlate with reduced risk of diabetes and cardiovascular disease onset and promote glucose and lipid control [7-13]. Healthful and macronutrient balanced vegan diets are naturally high in dietary soluble fibers, well- known for a multitude of health promoting benefits, such as glucose and lipid control [14-16]. Similarly, animal-based diets that include a high intake of soluble fiber containing fruits and vegetables, while minimizing red meat intake can also be health promoting. We previously studied the metabolic non-equivalence between balanced vegan and animal diets in healthy subjects that mediated an insulin, lipid and amino acid signature at fasting known to be associated with diabetes risk after 48 hours [17].

Postprandial studies permit the comprehensive examination of complex interactions between the food matrices, components and human metabolism after meal ingestion to shed light on metabolic response and adaptation [18]. Such research is fundamental to substantiate personalized nutritional approaches. For example, postprandial lipaemia (hypertriglyceridemia) from a high fat meal, known to worsen in individuals with type 2 diabetes, is associated with cardiovascular disease risk and acute cardiovascular events [19-21]. Postprandial lipaemia can be lowered with higher intake of dietary fiber, polyphenols, medium chain fatty acids and long-chain n-3 polyunsaturated fatty acids [22-25]. Postprandial dysmetabolism is distinguished by elevated glucose and lipids and associated with the onset of cardiovascular events [26]. In fact, postprandial dysmetabolic responses are useful to demonstrate individual resilience to high fat and high glucose challenges, known as phenotypic flexibility [27-29] .

Comparison of postprandial responses to vegan and animal meals holds the potential to provide a deeper understanding of the cumulative impact of meals, snacks and timing on fasting results. For the present analysis, we investigated the quantitative and correlative impact of meal nutrient composition in vegan and animal diets on postprandial metabolic response as a follow-on analysis from the same study represented in our first publication. This was achieved by evaluating clinical variables, such as insulin, glucose, triglycerides, amino acid, fatty acid and bile acid responses to meals and snacks from each diet type. This investigation was conducted to describe which meal compositions contributed optimal clinical and metabolomic biomarker

results in order to make recommendations for nutrient composition improvement using either vegan or animal based diets.

EXPERIMENTAL SECTION

This study is a follow on analysis of postprandial results from a study previously published which evaluated the fasting results from the vegan and animal diet interventions on day 3 [17]. Further description of the methods can be found in **Chapter 2**.

Study Population and Ethical Approval

This study was conducted in accordance with the ethical principles of Good Clinical Practice and the Declaration of Helsinki, approved by the Ethical Committee of Lausanne University School of Medicine, Switzerland (CER-VD, ref no. 222/14), and registered on ClinicalTrials.gov with the identifier NCT02223585. All participants provided written informed consent for study participation and were offered financial compensation agreed by the ethical committee for time spent and schedule inconveniences.

A total of 56 healthy male and female volunteers were pre-screened at information sessions held at the Metabolic Unit, Nestlé Research Center (Lausanne, Switzerland). Out of the 32 participants who signed informed consent, 26 were enrolled in the study (6 screening failures), 5 dropped out and 21 healthy participants (10 men, 11 women) completed this pilot study (**Figure S1**). Two participants dropped out because of non-serious adverse events, and another three decided not to proceed with the study. All participants habitually ate a heterogeneous diet including animal and vegan proteins before entrance into the study.

Study inclusion criteria were age (from 18 to 55 years), regular bowel movement (at least once every 1-2 days), and body mass index (BMI, from 18.5 to 27 kg.m-2). Health status was assessed by a physician during a screening visit as a standard medical visit with blood chemistry analysis. Exclusion criteria included special diets (vegetarian, high protein, and low cholesterol or weight loss program), pregnancy, food allergy, smoking, high alcohol consumption (more than 2 drinks per day), and excessive physical exercise (more than 5 moderate physical exercises per week).

Diet Interventions

All meals and snacks were provided by the Metabolic Unit (MU). Participant compliance with breakfast, lunch, morning and afternoon snacks were supervised by

the MU. The energy provided by vegan and animal meals was personalized for each participant according to their calculated resting energy requirements from anthropometrics, gender and level of physical activity [30]. Macronutrient composition was matched for the day between animal and vegan-based menus, and was calculated based on 20% protein, 50% carbohydrate and 30% fat of total calories within ±5% of calculated needs of each participant. However, the macronutrient composition for the individual meals and snacks differed within and across diets even though total compositions were matched (**Table S1**).

Clinical Trial Design

The clinical trial was a randomized, open label, cross-over, controlled study. Study participants were randomly assigned to the animal and plant protein challenges using Medidata Balance with dynamic allocation [31].The study lasted five weeks following a one week run-in phase (Week 1 = W1) that defines baseline of the participant's normal diet and lifestyle. Participants were randomly assigned to either animal or vegan meals for three consecutive days (Tuesday, Wednesday and Thursday). During each 3-day intervention, participants ate the same meals on each day, including breakfast, morning snack, lunch, afternoon snack, and dinner (**Figure S2**). All meals and snacks were prepared and provided on site (MU) to study participants under supervision by the MU staff with the exception of the dinner meal, which was packaged for home consumption. Fasting analysis results of the 3 day interventions have been previously published [17]. This study analyzes the results from plasma drawn at 7 time-points on day 3 after breakfast and lunch, to evaluate post-prandial response of each diet type. The timeframes between meals and snacks were approximately 2 ½ hours between breakfast and the morning snack, 2 hours between the morning snack and lunch, and 4 hours between lunch and the afternoon snack. As indicated in **Figure 1,** the considered timepoints are: breakfast minus 15 minutes [T0], breakfast plus 1 hour [T1], breakfast plus 2 hours [T2], lunch minus 15 minutes [T3], lunch plus 1 hour [T4], lunch plus 2 hours [T5], lunch plus 4 hours [T6], and lunch plus 6 hours [T7].

Amino acids, bile acids, clinical biomarkers and metabonomics analyses

Small molecule analysis (amino acids, clinical biomarkers, and metabonomics) of plasma samples was conducted using the same methodologies previously reported [29] (see Methods Supplement). For bile acids analysis, a method providing a broader coverage of targeted biochemical species was employed, differing from the quantification of 18 major bile acids used for the fasting plasma sample analyses. Plasma samples were extracted and prepared according to previously published methods [32, 33]. Briefly, all standards were obtained from Steraloids Inc. (Newport, RI, USA) and TRC Chemicals (Toronto, ON, Canada), and 9 stable isotope-labeled bile acid standards

were used as internal standards. An ultra-performance liquid chromatography coupled to tandem mass spectrometry (UPLC-MS/MS) system (ACQUITY UPLC-Xevo TQ-S, Waters Corp., Milford, MA, USA) was used to quantitate bile acids in the human plasma Data acquisition was performed using MassLynx version 4.1 and quantification was performed using TargetLynx version 4.1 (Waters, Milford, MA, USA).

STATISTICAL ANALYSIS

Study of differences in diet intake at breakfast, lunch and dinner
Statistical significance of observed nutrient composition differences across meals and snacks was calculated using a Wilcoxon signed rank-test using the p value <0.05 as an initial threshold of significance. False Discovery Rate (FDR) <0.10 was used to further assess the statistical significance of the results.

Study of post-breakfast and post-lunch time diet effects in plasma using the AUC model
A linear mixed effect model was used to assess the association between genders, diet type and diet sequence with the total changes in metabolites before and after lunch. The model was fit separately for each metabolite and for each time-period. The total metabolite changes between post-breakfast (breakfast, morning snack) and post-lunch (lunch, afternoon snack) periods were assessed with Area Under the Curve (AUC). AUCs were computed using the trapezoidal rule and their normality was assessed with the Shapiro-Wilk test: those showing a Shapiro-Wilk p-value ≤ 0.05 were transformed using the Box-Cox method, prior to fitting the model. The carry-over effect was assessed, as done previously [29].

Study of time specific diet effects post-breakfast, post-lunch and fasting day 3 post-dinner day 2 in plasma using ANOVA
To compare the metabolic effects of the diet type at specific timepoints, the interaction between time and diet was fit for each metabolite with a linear mixed effect model. With an ANOVA test, gender, diet and diet sequence affected metabolic measurements at fasting. Thus, the mixed model was applied to concentration values previously corrected for the baseline (fasting visit at day 3 of diet). For metabolites showing a significant time/diet interaction term (p-value≤0.05), a post-hoc analysis was performed. Least squares means were used to determine the difference between the vegan and the animal (baseline) diet at each time point. False Discovery Rate (FDR) <0.10 was used to assess the statistical significance of the results.

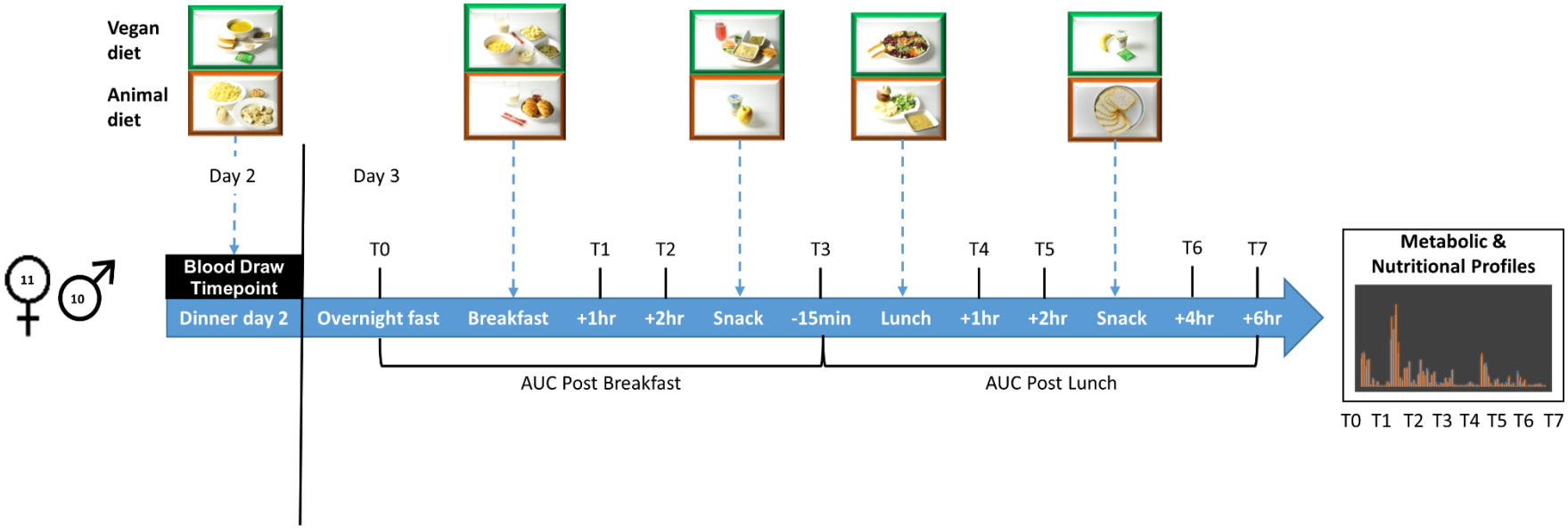

Figure 1. Schema. This randomized, controlled, cross-over pilot study compared 21 (10 men, 11 women) participants. Vegan and animal diets were tailored to individual kilocalorie needs and matched over the day for macronutrient composition but not between meals and snacks. Each diet type was fed for 3 days with 7 postprandial measurements on day 3 during breakfast and lunch. Clinical, amino acid, fatty acid and bile acid metabolic biomarkers were measured and compared at each timepoint and correlated with dietary intake. To Fasting = breakfast minus 15 min? T1 Breakfast +1 hour,T2 Breakfast +2 hours, T3 Lunch minus 15 minutes ,T4 Lunch + 1 hour, T5 Lunch + 2hours, T6 Lunch + 4 hours, T7 Lunch + 6 hours Note: post breakfast and post lunch snacks outside of 2 hour sampling timepoints. Post lunch snack generally 3-4 hours after lunch.

Correlation between dietary intake and metabolite levels

A paired Wilcoxon signed-rank test was used to investigate the relationships between the nutrient intakes on the concentration of plasma metabolites. For each meal, intakes were compared across diets, with results providing FDR q-value <0.10 considered significant. To better explore the results obtained with the AUC analysis, also the intakes of "combined meals" (breakfast+morning snack, lunch+afternoon snack, dinner+emergency snack) were compared across diets. The correlation between dietary intake and plasma metabolite levels was calculated with the Spearman correlation. Spearman's rho statistic was used to test the significance of the association. P-values were corrected for multiple testing using FDR <0.10.

RESULTS

Population Characteristics

A total of 21 (11 females, 10 males) healthy participants completed the clinical trial. Individuals were similar in age and BMI. Baseline characteristics have been described previously [29].

Meal and Snack Compositions

Supervised meal and snack nutrient compositions were analyzed from participant compliance records to quantify actual intake differences due to individual preferences, appetite and satiety. Of the 43 nutrients analyzed from the diet intervention intakes during dinner day 2, and breakfast and lunch day 3; we have highlighted 24 nutrient compositions for each meal and snack due to their significant influence on the postprandial metabolite response (**Table S1**). Statistical analysis was completed using mean nutrient intakes for: i) breakfast and morning snacks from day 3; ii) lunch and afternoon snacks from day 3; and, ii) dinner and emergency snacks from day 2 (**Table 1**).

All mean dinner and evening snack intakes from day 2 were statistically significantly greater from the animal diet with the exception of percent kilocalories (kcal) from protein (PROT %), total dietary fiber (TDF), percent kilocalories from carbohydrate (CHO %), percent kilocalories from fat (Fat %) and polyunsaturated fatty acids (PUFA). Particularly notable was the large difference in kcal (915.24 vs. 400.10 kcals in animal vs. vegan diets) and protein (PROT) (63.18 vs. 21.74 g in the animal vs. vegan diets). The vegan diet maintained statistically significantly higher TDF and PUFA contents (**Table 1, Table S1**).

Breakfast and morning snack intakes from day 3 (e.g. Kcals, CHO, PROT, AA, fat) were statistically significantly greater for the vegan diet with the exception of saturated fatty acids (SFA) which were statistically significantly greater in the animal diet (FDR<0.10). Lysine (LYS), methionine (MET), proline (PRO) and CHO % differences did not reach statistical significance (**Table 1, Table S1**).

Lunch and afternoon snack intakes were statistically significantly higher from the animal diet for Kcal, fat, fat %, MUFA, PUFA, PROT, PROT % and 9 amino acids (AA). Total dietary fiber and methionine contents were higher from the vegan diet and statistically significant (**Table 1, Table S1**).

Clinical Response to Meal and Snack Compositions

Out of the 24 metabolic parameters analyzed, statistically significant differences were observed in insulin, triglycerides and glucose AUCs between diets. Insulin, triglycerides and glucose AUCs were significantly elevated from the vegan diet during the post-breakfast timeperiod. Insulin decreased on the vegan diet and became elevated from the animal diet during the lunch period. Glucose remained significantly elevated from the vegan diet during the post-lunch timeperiod (**Figure 2, Table S2**). Temporal analysis using ANOVA (timepoints To-T7) revealed statistically significant differences at T1, T2 and T7 for triglycerides (TGs) with the elevated TGs from the vegan diet being most notable at T1 and T2. A part from T1, all the timepoints were statistically significant for insulin, with T4 most notable for an elevated peak from the animal diet. Timepoint T3 was statistically significant for glucose and the elevation between T3 and T4 is most notable (**Table S3**). Insulin and TG responses showed gender dimorphism (see **gender dimorphism supplement, Table S4**).

The elevated TG and insulin from the vegan breakfast demonstrated a strong statistically significant correlation between the TG and insulin AUCs, and CHO intake, elevated from the vegan diet (**Table 2, Figure S4**).

The prolonged elevated glucose from the vegan diet at lunch correlated significantly with TDF, suggesting a correlation with slowed absorption of glucose over time. Fasting plasma day 3 insulin was significantly inversely correlated with TDF intake from dinner day 2. In this case, insulin had been elevated from the animal diet and fiber intake was higher from the vegan diet [29] (**Table 2, Figure S5**).

Elevated triglycerides and insulin from the vegan breakfast demonstrated a significant correlation with their respective AUCs for total fat intake, consistent with the higher fat intake from the vegan breakfast (**Table 1, Table 2, Figure S4**).

Table 1. Significant diet intake differences between AUC timeperiods.

		Means						Significance					
Nutrient		**PB**	**PB Vegan**	**PL Animal**	**PL**	**PD**	**PD**	**P value**	**FDR PB**	**P value**	**FDR PL**	**P value**	**FDR PD**
Kilocalories	**Kcal**	598.35	895.51	674.21	556.26	915.24	400.10	**1.91E-06**	**5.15E-05**	**1.22E-03**	**3.30E-02**	**6.40E-05**	**1.73E-03**
Protein (g)	**PROT**	15.89	36.80	27.92	22.23	63.18	21.74	**6.40E-05**	**1.73E-03**	**3.42E-04**	**9.23E-03**	**6.40E-05**	**1.73E-03**
Percent protein	**PROT %**	23.07	33.32	28.60	31.10	26.51	21.25	**1.91E-06**	**5.15E-05**	**1.37E-03**	**3.70E-02**	**1.81E-02**	4.89E-01
Alanine (mg)	**ALA**	665.18	1389.77	1230.18	1004.30	3294.94	870.64	**6.40E-05**	**1.73E-03**	**7.44E-04**	**2.01E-02**	**6.40E-05**	**1.73E-03**
Arginine (mg)	**ARG**	689.86	2392.93	1271.14	1501.08	3674.33	1681.07	**6.40E-05**	**1.73E-03**	**7.81E-03**	2.11E-01	**6.40E-05**	**1.73E-03**
Cysteine (mg)	**CYS**	206.14	472.50	437.60	213.29	992.49	240.14	**6.40E-05**	**1.73E-03**	**6.37E-05**	**1.72E-03**	**6.40E-05**	**1.73E-03**
Glycine (mg)	**GLY**	442.16	1185.10	1250.76	949.95	3026.74	807.36	**6.40E-05**	**1.73E-03**	**1.51E-04**	**4.07E-03**	**6.40E-05**	**1.73E-03**
Histidine (mg)	**HIS**	403.89	754.92	718.19	635.98	1945.85	497.95	**6.40E-05**	**1.73E-03**	**2.61E-02**	7.04E-01	**6.40E-05**	**1.73E-03**
Isoleucine (mg)	**ILE**	862.68	1259.65	1110.30	932.10	3372.88	854.80	**3.43E-04**	**9.27E-03**	**3.12E-03**	**8.43E-02**	**6.40E-05**	**1.73E-03**
Leucine (mg)	**LEU**	1582.60	2221.90	1921.19	1173.86	5139.06	1220.38	**2.62E-04**	**7.09E-03**	**6.37E-05**	**1.72E-03**	**6.40E-05**	**1.73E-03**
Lysine (mg)	**LYS**	1164.13	1372.62	1359.37	1334.99	4604.08	1169.70	**2.39E-02**	6.44E-01	6.76E-01	1.00E+00	**6.40E-05**	**1.73E-03**
Methionine (mg)	**MET**	422.46	475.89	491.77	299.85	1593.38	244.21	**4.38E-02**	1.00E+00	**6.37E-05**	**1.72E-03**	**6.39E-05**	**1.72E-03**
Phenylalanine (mg)	**PHE**	844.86	1337.51	1049.30	1056.30	2932.96	957.98	**9.90E-05**	**2.67E-03**	8.62E-01	1.00E+00	**6.40E-05**	**1.73E-03**
Proline (mg)	**PRO**	1747.60	1454.31	1540.73	626.24	4356.06	734.66	**4.37E-03**	1.18E-01	**6.37E-05**	**1.72E-03**	**6.40E-05**	**1.73E-03**
Serine (mg)	**SER**	966.74	1435.11	1180.25	1175.40	1948.72	1027.00	**1.74E-04**	**4.70E-03**	8.35E-01	1.00E+00	**6.40E-05**	**1.73E-03**
Threonine (mg)	**THR**	685.98	1124.94	907.19	866.57	2713.07	803.98	**8.57E-05**	**2.31E-03**	3.66E-01	1.00E+00	**6.40E-05**	**1.73E-03**
Tryptophan (mg)	**TRP**	193.17	267.54	335.40	255.04	894.69	184.07	**3.92E-04**	**1.06E-02**	**1.51E-04**	**4.07E-03**	**6.40E-05**	**1.73E-03**
Tyrosine (mg)	**TYR**	746.24	940.77	822.38	650.39	2081.37	677.18	**3.50E-03**	**9.46E-02**	**3.41E-04**	**9.22E-03**	**6.40E-05**	**1.73E-03**
Valine (mg)	**VAL**	1065.40	1436.82	1221.48	1056.06	3336.85	1022.83	**5.09E-04**	**1.37E-02**	**6.69E-03**	1.81E-01	**6.40E-05**	**1.73E-03**
Carbohydrate (g)	**CHO**	68.57	97.64	73.31	81.83	119.14	47.10	**6.40E-05**	**1.73E-03**	7.62E-02	1.00E+00	**6.40E-05**	**1.73E-03**
Percent carbohydrate	**CHO %**	92.40	92.45	115.98	120.46	50.80	47.70	6.09E-01	1.00E+00	3.44E-01	1.00E+00	3.66E-01	1.00E+00
Total fiber (g)	**TDF**	4.65	15.14	6.80	14.72	6.03	8.80	**6.37E-05**	**1.72E-03**	**6.37E-05**	**1.72E-03**	**1.38E-03**	**3.74E-02**
Fat (g)	**Fat**	27.31	36.90	29.15	14.63	17.10	13.30	**1.99E-04**	**5.38E-03**	**6.36E-05**	**1.72E-03**	**2.79E-03**	**7.54E-02**
Percent fat	**Fat %**	79.45	66.83	54.41	45.44	19.10	30.13	**6.68E-06**	**1.80E-04**	**1.81E-03**	**4.88E-02**	**2.18E-02**	5.88E-01
Polyunsaturated	**PUFA**	1.02	13.20	9.16	8.12	5.03	7.45	**6.40E-05**	**1.73E-03**	1.80E-01	1.00E+00	**1.08E-03**	**2.93E-02**
Monounsaturated	**MUFA**	5.99	15.98	11.83	4.35	6.94	1.98	**7.37E-05**	**1.99E-03**	**6.35E-05**	**1.72E-03**	**6.35E-05**	**1.71E-03**
Saturated fatty acids	**SFA**	18.32	6.81	7.84	2.14	3.56	2.59	**6.40E-05**	**1.73E-03**	**6.37E-05**	**1.72E-03**	**1.72E-02**	4.65E-01

The p values were calculated by performing a Wilcoxon signed rank-test. PB, post-breakfast; PL, post-lunch; PD, post-dinner. Significant p values in bold ($p<0.05$). Significant values in bold that meet false discovery rate FDR<0.1

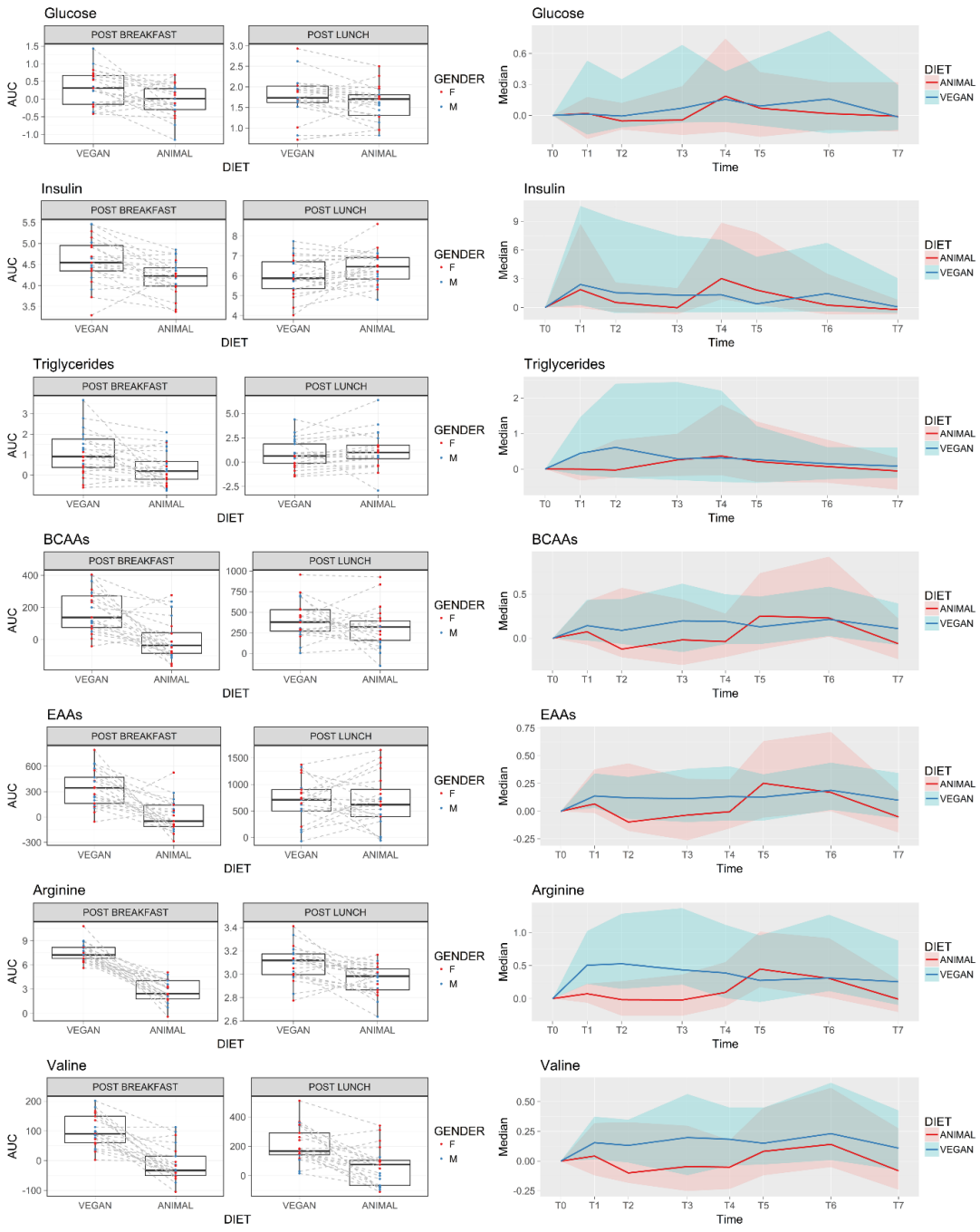

Figure 2. Clinical biomarkers TG, insulin, glucose changes according to meal composition timing. Amino acids changes according to meal composition timing. Figures on the left represent AUC changes across diets and meal period. Gender differences are shown with red (female) and blue (male) dots. Figures on the right display timepoint changes, with blue and red lines represent respectively the vegan and the animal diet. To Fasting = breakfast minus 15 min? T1 Breakfast +1 hour T2 Breakfast +2 hours T3 Lunch minus 15 minutes T4 Lunch + 1 hour T5 Lunch + 2hours T6 Lunch + 4 hours T7 Lunch + 6 hours Note: post breakfast and post lunch snacks outside of 2 hour sampling timepoints. Post lunch snack generally 3-4 hours after lunch. PB, post breakfast; PL, post lunch. A) glucose PB FDR=1.29E-02, PL FDR=5.60E-02; B) glucose T3 FDR<0.10; C) insulin PB FDR=6.54E-03, PL FDR<=5.60E-02; D) insulin T2-T7 FDR<0.10; E) triglycerides PB FDR=8.70E-03, PL FDR=3.75E-01; F) triglycerides T1-T2,T7 FDR<0.10; G) BCAAs (branch chain amino acids) PB FDR=2.09E-03, PL FDR=3.46E-01; H) branch chain amino acids T2-T3,T7; I) EAAs (essential amino acids) PB FDR=1.69E-04, PL FDR=9.48E-01; J) essential amino acids T2-T5,T7; K) arginine PB FDR=2.98E-09, PL FDR=1.57E-02; L) arginine T1-T5,T7; M) valine PB FDR=1.65E-04, PL FDR<=1.38E-03; N) valine T1-T5,T7.

Elevated glucose from the vegan lunch demonstrated a significant inverse correlation with the lunch AUC for total fat intake, consistent with the significantly lower fat intake from the vegan lunch (**Table 1, Figure S4**). Elevated triglycerides, insulin and glucose from the vegan breakfast demonstrated significant positive correlations with the breakfast AUC for total PUFA intake, found to be significantly elevated in the vegan breakfast (**Table 2, Figure S4**). Insulin and TG, elevated in plasma fasting day 3 from the animal dinner day 2 [29], inversely correlated with PUFA intake, consistent with the higher PUFA intake from the vegan dinner (**Table 1, Table 2**).

Plasma Amino Acid Response to differences in sources and Timing of Dietary Protein
From a targeted quantification of 21 amino acids (AA), a total of 14 individual amino acids (arginine, ornithine, phenylalanine, asparagine, valine, tryptophan methionine, proline, citrulline, lysine, leucine, isoleucine, threonine, histidine), including the branched chain amino acids (BCAAs) and essential amino acids (EAA), demonstrated statistically significant higher AUCs from the vegan diet during the breakfast timeperiod. This result reflects the higher intake of BCAAs from the vegan protein hemp supplement and the extremely low intake of foods rich in protein on the animal diet. Proline demonstrated significantly higher AUCs at breakfast and lunch from the naturally high proline animal diet. Valine) continued with a statistically significantly higher AUC from the vegan breakfast to lunch timeperiods despite the high protein animal lunch. Three AAs (lysine, methionine and proline) demonstrated significantly higher AUCs from the animal lunch timeperiod reflecting the differences in AA protein compositions between the two diet types (**Figure 2, Figure S3, Table S2**). All 21 amino acids demonstrated statistically significant timepoint interactions. Significant peaks can be easily visualized in **Figure 2**, especially between T4 and T5 for the animal diet. These same types of peaks were not visualized in the vegan diet in which the AAs, particularly the EAAs, appear more stable (**Figure 2, Table S3, Figure S3**). Amino acid responses showed gender dimorphism (See **gender dimorphism supplement**, **Table S4**).

A total of 11 plasma AA (arginine, proline, alanine, phenylalanine, valine, leucine, tryptophan, isoleucine, lysine, threonine, methionine) demonstrated statistically significant correlations with breakfast and lunch amino acid intakes. Protein intake was strongly and statistically significantly correlated with elevated total plasma BCAA and EAA AUCs from the high protein, vegan breakfast (FDR<0.10) (**Table 2, Figure S4**) Of the plasma AAs found to be statistically significant in a previous publication [29], EAAs and BCAAs showed a strong, statistically significant correlation with the high protein intake from the animal dinner on day 2 and seven individual plasma AAs significantly correlated with their respective AA intakes, from the animal diet dinner (**Table 2, Table 1**).

Table 2. Spearman's rank correlations (r) between plasma concentrations and dietary nutrient intakes for breakfast

Plasma	Diet	r PB	*P* value	r PL	*P* value	r FD3	*P* value
Amino acids							
ARG	ARG	0.75	**8.46E-09**	0.42	**5.94E-03**	0.09	5.75E-01
BCAAs	PROT %	0.70	**2.94E-07**	0.67	**2.72E-06**	0.28	6.93E-02
EAAs	PROT %	0.66	**1.81E-06**	0.69	**7.33E-07**	0.32	4.04E-02
EAAs	PROT	0.68	**9.04E-07**	-0.31	4.47E-02	0.72	**8.61E-08**
BCAAs	PROT	0.65	**3.34E-06**	-0.30	4.99E-02	0.62	**1.05E-05**
PRO	PRO	0.67	**1.51E-06**	0.11	4.75E-01	0.74	**2.60E-08**
ALA	ALA	0.54	**2.52E-04**	0.19	2.20E-01	0.38	1.22E-02
PHE	PHE	0.50	**8.60E-04**	-0.01	9.28E-01	0.33	3.48E-02
VAL	VAL	0.49	**9.31E-04**	-0.27	8.93E-02	0.67	**1.06E-06**
LEU	LEU	0.41	**6.29E-03**	-0.54	**2.51E-04**	0.54	**2.34E-04**
TRP	TRP	0.42	**6.13E-03**	-0.18	2.41E-01	0.44	**3.28E-03**
ILE	ILE	0.41	**7.32E-03**	-0.18	2.43E-01	0.36	1.85E-02
LYS	LYS	0.35	**2.33E-02**	0.05	7.76E-01	0.58	**6.51E-05**
THR	THR	0.33	**3.55E-02**	-0.09	5.50E-01	0.22	1.65E-01
MET	MET	0.33	**3.40E-02**	0.09	5.74E-01	0.46	**2.33E-03**
CYS	CYS	0.26	1.00E-01	0.43	**5.02E-03**	0.06	7.17E-01
GLY	GLY	-0.14	3.84E-01	-0.38	**1.44E-02**	0.06	7.07E-01
Fatty acids							
Capric acid (C10:0)	PUFA	-0.77	**2.26E-09**	0.16	3.09E-01	0.27	8.44E-02
Dodecanoic acid (C12:0)	PUFA	-0.74	**2.81E-08**	0.11	4.78E-01	0.50	**7.37E-04**
Myristic acid (C14:0)	PUFA	-0.67	**1.55E-06**	-0.01	9.68E-01	0.22	1.59E-01
Caprylic acid (C8:0)	PUFA	-0.52	**4.05E-04**	0.28	6.79E-02	0.10	5.41E-01
Pentadecanoic acid (C15:0)	PUFA	-0.59	**3.78E-05**	-0.17	2.85E-01	0.15	3.52E-01
5-dodecanoic acid (C12:1)	PUFA	-0.47	**1.48E-03**	0.06	7.23E-01	0.45	**2.59E-03**
Myristoleic acid (C14:1)	PUFA	-0.38	**1.20E-02**	-0.02	8.89E-01	0.29	5.79E-02
Heptadecanoic acid (C17:1)	PUFA	-0.32	**4.20E-02**	-0.20	2.13E-01	0.20	1.99E-01
Capric acid (C10:0)	SFA	0.73	**3.47E-08**	0.62	**1.10E-05**	-0.20	1.99E-01
Dodecanoic acid (C12:0)	SFA	0.61	**1.50E-05**	0.63	**9.28E-06**	-0.17	2.85E-01
Myristic acid (C14:0)	SFA	0.55	**1.78E-04**	0.56	**1.20E-04**	-0.16	3.07E-01
Caprylic acid (C8:0)	SFA	0.60	**2.94E-05**	0.48	**1.38E-03**	-0.08	6.25E-01
Pentadecanoic acid (C15:0)	SFA	0.36	**1.87E-02**	0.37	**1.47E-02**	-0.06	6.94E-01
5-dodecanoic acid (C12:1)	SFA	0.32	**4.16E-02**	0.51	**4.84E-04**	-0.01	9.38E-01
Clinical biomarkers							
Insulin	CHO	0.45	**2.60E-03**	0.23	1.35E-01	0.11	4.82E-01
TG	CHO	0.74	**1.84E-08**	0.42	**5.26E-03**	0.34	2.83E-02
TG	CHO %	-0.32	**3.81E-02**	0.28	7.87E-02	0.24	1.31E-01
TG	FAT	0.72	**7.12E-08**	-0.01	9.33E-01	0.04	7.81E-01
Glucose	FAT	0.16	3.13E-01	-0.46	**2.13E-03**	0.16	3.00E-01
Insulin	FAT	0.41	**7.31E-03**	-0.13	4.06E-01	-0.21	1.78E-01
Insulin	FAT %	-0.32	**3.92E-02**	-0.21	2.02E-01	-0.18	2.62E-01
TG	PUFA	0.62	**1.42E-05**	0.13	3.98E-01	-0.33	3.33E-02
Insulin	PUFA	0.44	**3.16E-03**	0.06	7.09E-01	-0.50	**7.10E-04**
Glucose	PUFA	0.32	**3.89E-02**	-0.06	7.13E-01	0.03	8.42E-01
Glucose	SFA	-0.26	1.02E-01	-0.51	**5.65E-04**	0.18	2.60E-01

Table 2. Spearman's rho statistic was used to test the significance of the association PB, post-breakfast; PL, post-lunch; FD3, fasting day 3; r, correlation coefficient. Conventional p values are shown and significant false discovery rates in bold (FDR<0.10). Kcal, kilocalories; PROT, protein, PROT%, percent kilocalories from protein; ARG, arginine; BCAAs, branched chain amino acids; EAAs, essential amino acids; PRO, proline; ALA, alanine; PHE, phenylalanine; VAL, valine; LEU, leucine; TRP, tryptophan; ILE, isoleucine; LYS, lysine; THR, threonine; MET, methionine; CYS, cysteine; GLY, glycine; PUFA, polyunsaturated fatty acids; SFA, saturated fatty acids; TG, triglycerides; CHO, carbohydrates; CHO%, percent kilocalories from carbohydrates; TDF, total dietary fiber.

Plasma Bile Acid Response to Composition Differences and Timing of Meals and Snacks

We analyzed 38 plasma bile acids for a comprehensive view of the postprandial phase response [29]. From this analysis, three primary bile acids demonstrated statistically significant AUC changes during the breakfast and lunch periods. Cholic acid (CA) demonstrated statistically significantly higher AUCs and □-Chenodeoxycholic acid (bCDCA) demonstrated statistically significantly lower AUCs from the vegan diet during breakfast and lunch. The chenodeoxycholic acid (CDCA) AUC was statistically significantly higher from the vegan diet during lunch only. Ten secondary bile acids showed statistically significant AUC changes. Four of these (12-Ketolithocholic acid [12-ketoLCA], 7-Ketolithocholic acid [7-ketoLCA], Glycolithocholic acid-3-sulfate [GLCA-3S] and, Lithocholic acid-3-sulfate [LCA-3S]) significantly increased from the vegan diet after breakfast and lunch. Four tertiary bile acids demonstrated statistically significant AUC changes from lunch only (**Figure 3, Table S2**). Analyses of timepoint results (ANOVA, To-T7) revealed 5 primary, 14 secondary, and 8 tertiary bile acids with complex temporal profiles (**Table S3, Figure S6**).

Of the bile acids with statistically significant AUCs, TDF was positively correlated with GLCA-3S at breakfast and lunch, but not dinner; The KCAL, CHO, TDF, PROT, 14 AAs, fat, PUFA and MUFA with positive correlations with bile acids (GLCA-3S and LCA-3S) at breakfast, were reversed at lunch, reflecting the change in relative concentrations of the vegan diet (**Figure 4, Table 1**).

Plasma Fatty Acid Response to Sources and Timing of Dietary Fat Intake
Out of 31 fatty acids analyzed, a total of 7 (5-dodecanoic, capric, caprylic, dodecanoic, 1,2-methylpentanoic, myristic, and pentadecanoic acids), showed elevated AUC from the animal diet during the breakfast period. Of those, AUC of capric, dodecanoic, myristic and pentadecanoic acids remained elevated from the animal diet during the lunch period.

AUC of eicosenoic and myristoleic acids were statistically significantly elevated from the animal diet during the lunch period only. Temporal analysis using ANOVA further revealed 8 fatty acids with statistically significant timepoint differences across the two diets (vegan vs. animal). Of note, caprylic acid was elevated at T2 (breakfast period) and myristoleic acid was higher at T5. (**Figure 5, Table S3**).

Dietary intake of saturated fatty acids (SFA), elevated on the animal diet, demonstrated statistically significant correlations with 6 plasma fatty acids (capric, dodecanoic, myristic, caprylic, pentadecanoic, and 5-dodecenoic acids) during the breakfast and lunch timeperiods (**Table 2, Table 1, Figure S4, Figure S5, Table 1**).

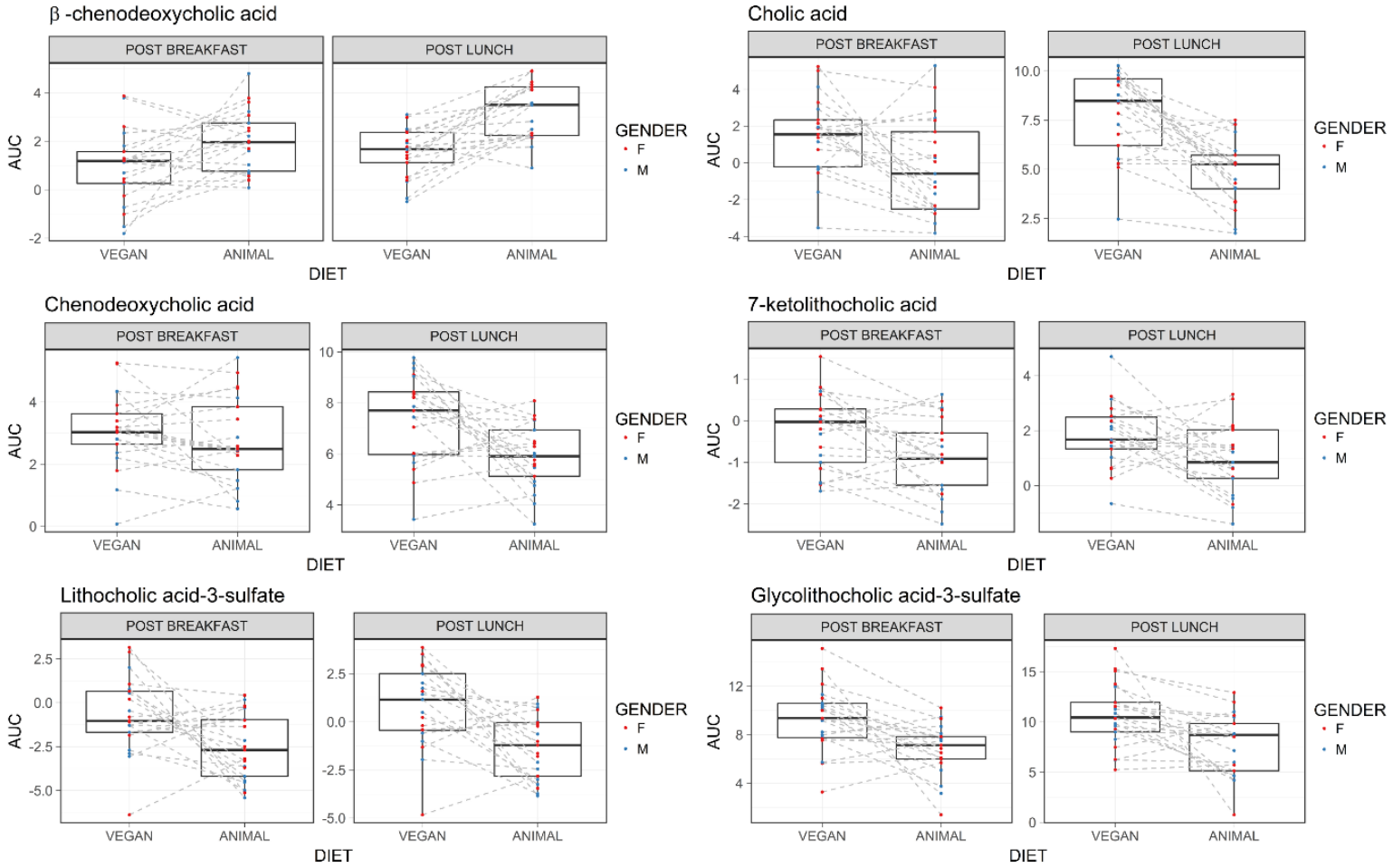

Figure 3. Representative bile acids that showed statistically significant different AUC responses post-breakfast and post-lunch to the vegan vs. animal diets are graphically depicted. Gender differences are shown with red (female) and blue (male) dots. β-chenodeoxycholic acid post-breakfast FDR=8.74E-02, post-lunch FDR=5.53E-04; Cholic acid post-breakfast FDR=8.68E-02, post-lunch FDR=5.52E-04; Chenodeoxycholic acid post-breakfast FDR=7.4E-01, post-lunch FDR=2.85E-02; 7-ketolithocholic acid post-breakfast FDR=8.68E-02, post-lunch FDR=6.12E-02; Lithocholic acid 3 sulfate post-breakfast FDR=8.44E-02, post-lunch FDR=1.42E-02; Gglycholithocholic acid post-breakfast FDR=8.68E-02, post-lunch FDR=8.88E-01.

DISCUSSION

The metabolic impact of vegan and animal meals and snacks was compared in a cross-over study design. Daily intakes were matched for energy densities of CHO, PROT and fat (50%, 20%, and 30%). The vegan diet contained approximately twice the amount of TDF than the animal diet (39 and 18 grams respectively). The macronutrient and TDF compositions of the vegan diet were similar to the Nordic diet (51% CHO,17% PROT, 32% fat, 41g TDF) [34]. Meals and snacks were not matched for macronutrient composition. This allowed us to observe the limitations, benefits and opportunities to improve metabolic signatures with both diet types. Comparison of postprandial signature responses to fasting signatures previously published on this cohort provided a deeper understanding of the cumulative impact of meals, snacks and timing on fasting results [29].

Lipid and glycemic responses are elevated from both animal and vegan meal combinations
Postprandial TG and insulin are known to be higher in individuals with coronary artery disease and may play a role in the development of atherosclerosis, a risk factor for cardiovascular disease [35, 36]. Hypertriglyceridemia is also a common abnormality observed in obesity, metabolic syndrome and diabetes [37]. In a previous paper on this same cohort, fasting results after 48 hours of following the supervised diets demonstrated lower TG and insulin plasma levels from the vegan diet. In the present study, the higher kcals, CHO and fat from the vegan breakfast produced elevated plasma TG, insulin and glucose AUCs (**Figure 2, Table S2**). The peak in TG from the vegan diet two hours after breakfast (T2) reflected the high CHO content of the muesli and high fat content of the cashew butter (**Table S1**).

Glucose remained mildly elevated with a peak in glucose and insulin 4 hours post-lunch (T6), reflecting the trend toward higher CHO and TDF intakes from the vegan beans, rice and banana snack (**Figure 2, Figure S2**).

The continual rise in glucose from 2 hours after breakfast (T2) until 1-hour post-lunch (T4), may have been a remnant from the sugar contained in the drink taken during the morning snack on the vegan diet mixed with the slowed digestion and short chain fatty acid production from the soluble dietary fiber (**Figure 2**). This would have sustained glucose levels and prevented the large plasma glucose peak often seen after fast acting sugar intake [38-40]. Vegan diet soluble fiber sources included hemp protein powder, hummus, red beans, banana and lentils (**Figure S2**). The glucose and insulin peaks visualized from the animal diet at T4 (1 hour post-lunch) reflected the higher saturated fat intake from the hamburger known for its association with inflammation and insulin resistance (**Figure 2, Table S3**) [41].

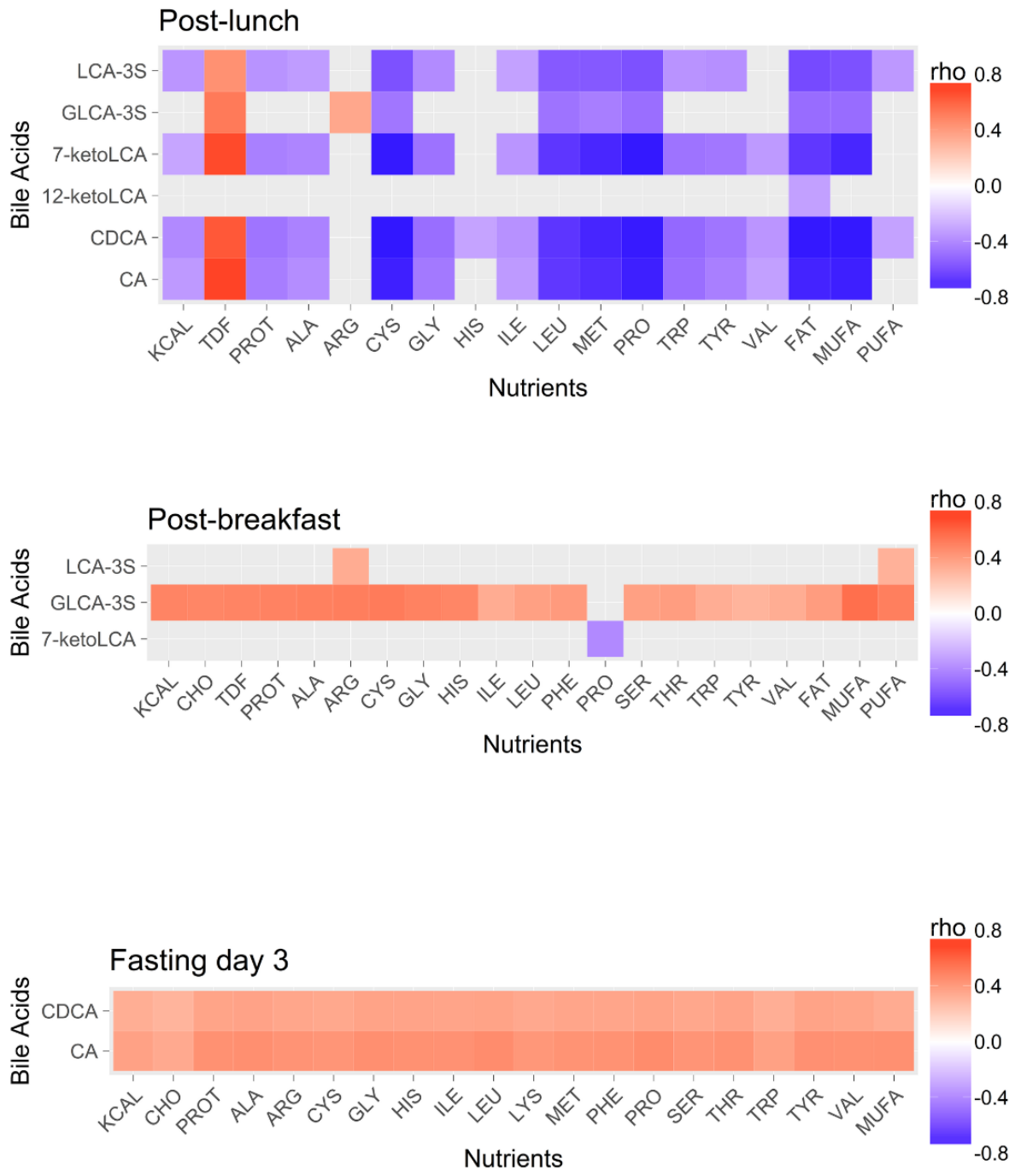

Figure 4. (A-C) Spearman correlations between significant plasma bile acids and diet intake are graphically represented with a heatmap for the post-breakfast, post-lunch and dinner day 2 AUC responses. KCAL, kilocalories; TDF, total dietary fiber; PROT, protein,; ALA, alanine; ARG, arginine; CYS, cysteine; GLY, glycine; HIS, histidine; ILE, isoleucine; LEU, leucine; MET, methionine; PRO, proline; TRP, tryptophan; TYR, tyrosine; VAL, valine; FAT, total fat; MUFA, monounsaturated fatty acids; PUFA, polyunsaturated fatty acids; CHO, carbohydrate; PHE, phenylalanine; SER, serine; THR, threonine.

Together, these results demonstrated the immediate metabolic influence of alterations in nutrient combinations of both diet types.

Postprandial amino acid plasma response varies with dietary AA composition irrespective of diet type

Elevated AA concentrations are associated with higher risk of insulin resistance and obesity [29, 42]. In a previous publication, we demonstrated the association of a suboptimal BCAA associated metabolic signature at fasting after 2 days following the supervised animal based protein diet [29]. Additionally, postprandial BCAAs were previously associated with fasting glucose and insulin concentrations [43]. In the present study, significantly elevated plasma AAs from the vegan diet breakfast resulted from the hemp protein supplement and produced a similar AA signature to the 2 day animal diet response suggesting a large difference in protein intake from either animal or vegan food sources could produce the same, suboptimal metabolic signature (**Figure 2, Figure S3, Table S2**).

Strong correlations in the data between plasma AA concentrations and dietary AA intake substantiate the rapid and direct impact of dietary intake on metabolism (**Table 2, Figure S4, Figure S5**). Plasma lysine, methionine and proline were elevated after the animal diet lunch due to significant concentrations in the hamburger; and may have accumulated from the morning yogurt animal diet snack. Notably, the BCAAs were not elevated, despite their large quantity in the animal diet foods, due to the addition of BCAAs in the form of Hemp protein powder to the vegan diet (**Figure S5, Table S2, Figure S2**). Dietary methionine may worsen insulin sensitivity while lysine attenuates glucose response in the absence of any effect on insulin [44, 45]. Thus, the insulin peak 1 hour after the animal lunch in the absence of a significant glucose peak may have been influenced by the lysine and methionine content of the hamburger lunch (**Figure 2, Figure S2).**

Bile acids are elevated and absorption is prolonged from the TDF content of the vegan meals

Bile acids facilitate postprandial lipid digestion, transport and metabolism. As nutrient signaling hormones, they interact with insulin to regulate nutrient metabolism in the liver. Elevated bile acid concentrations are associated with improved glucose homeostasis and lipid profiles [46-48].

The majority of the bile acids were elevated after the vegan breakfast (secondary bile acids) and lunch timeperiods (primary and secondary bile acids) (**Figure 3, Figure S6, Table S2**). Dietary fiber intake slows CHO, AA and lipid absorption reducing the risk of

hyperlipidemia, hypercholesterolemia and hyperglycemia [51]. It also shifts gut microbial populations by facilitating bacterial fermentation [7]. Significant bile acid correlations were observed with TDF across these two timeperiods suggesting TDF may have had the biggest impact on the bile acid concentrations, particularly with GLCA-3S, a conjugated secondary bile acid from bacterial colonic activity (**Figure 4**). It is possible that the additional presence of the TDF, prominent in the vegan diet, facilitated GLCA-3S colonic conjugation causing its significant elevation from the vegan diet. The elevated cholic acid observed at both mealtimes, with a significant peak 4 hours post-lunch (T6), may be associated with increased energy expenditure and could be related to the decreased body mass index seen in vegetarians (**Figure 3, Table S2, Table S3**). Further research is needed to explore this potential effect in a postprandial state [52, 53].

Elevated plasma fatty acid concentrations correlate with saturated fatty acid intake from the animal breakfast and lunch timeperiods

Saturated fats are known to be hypercholesterolemic and insulin resistance promoting relative to their less saturated counterparts [41, 54]. However, certain saturated fats have health benefits. Capric and caprylic acid are both saturated fats and medium chain fatty acids that may reduce plasma cholesterol through its excretion and are inversely correlated with pancreatic cancer and ischemic heart disease [55-57]. Saturated fatty acid dietary intake was significantly greater for the animal diet and positively correlated with elevated plasma fatty acid concentrations for both breakfast and lunch timeperiods (**Table 1, Table 2, Table S2**).

The large capric acid plasma concentration seen in participants on the animal diet is consistent with the high capric acid intake from animal fats, such as the butter in the croissant, yogurt and hamburger eaten during the breakfast and lunch periods on the animal diet (**Figure 5, Figure S2, Table S3**). Coconut milk fed to the study participants on the vegan diet at dinner also produced elevated fasting plasma capric acid the morning after, further substantiating the rapid sensitivity of plasma fatty acid response to a well-controlled diet [29].

Caprylic acid is found in animal fats in smaller quantities than capric acid as reflected in the time-point fatty acid variation (**Figure 5**) where we can visualize a 2 hour post-breakfast (T2) peak that reflects the butter content of the croissant and milk from the animal diet breakfast (**Figure 5**). The 5-dodecenoic acid (monounsaturated form of dodecanoic acid) peak reflects butter and milk in the animal diet breakfast as well (Figur**e 5, Figure S2**). Coconut is also a good source as reflected in the post dinner fasting results previously reported [29]. Myristoleic acid, the monounsaturated form of

myristic acid, showed strong peaks reflecting the fat content of the animal diet at the breakfast and lunch meals and seemed to fall quickly in between meals. It appears the lunch-time hamburger meat had the largest impact on the myristoleic acid (**Figure 5, Table S3, Figure S2**).

Here we highlight the significantly increased fatty acids from the animal diet and potential health benefits. However, in our previous publication on fasting results from this study, we observed elevated fatty acids from the coconut milk used in the dinner meal on the vegan diet [29]. The results in this paper, when combined with the former, suggest that optimal healthy fatty intake can be achieved from either a carefully planned vegan or animal diet. A combination of the two options in a flexitarian approach may be optimal for health maintenance to prevent the accumulation of longer chain SFAs that present more health challenges [41, 54].

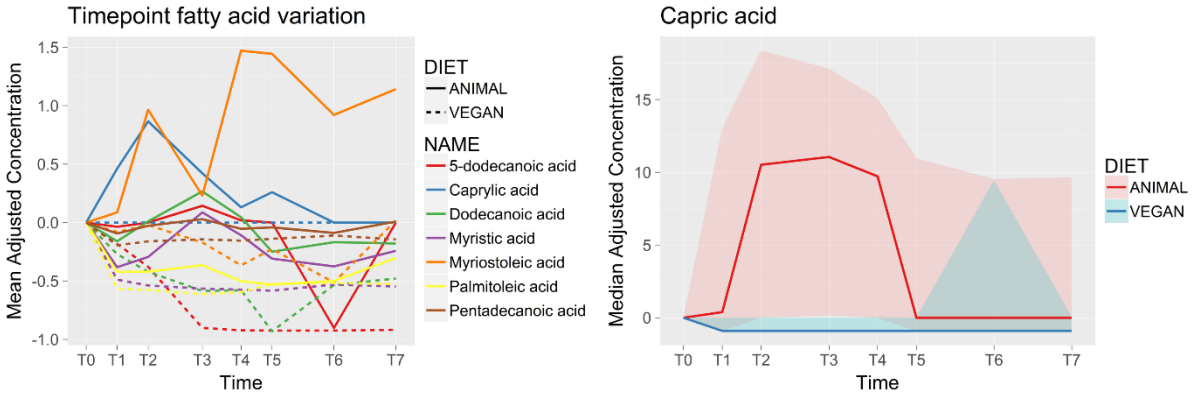

Figure 5. Fatty acid timepoint variation. All fatty acids with significant timepoint variations are graphically depicted. To compare the metabolic effects of the diet type at specific time-points, the interaction between time and diet was fit for each metabolite with a linear mixed effect model. 5-dodecanoic acid FDR<0.10 for T2-T7; caprylic acid FDR<0.10 for T1-T5; dodecanoic acid FDR<0.10 for T2-T7; myristic acid FDR<0.10 T1-T7; myristoleic acid FDR<0.10 for T2-T7; pentadecanoic acid FDR<0.10 for T1-T5,T7; palmitoleic acid FDR<0.10 for T1-T4,T7; capric acid FDR<0.10 for T1-T7.

Study limitations and Opportunities

Carbohydrate, fat and protein macronutrient compositions were matched across diet types for daily intake but not between individual meals and snacks. If macronutrient compositions were matched at meal times, investigation of micronutrient impact on metabolic health signatures of different diet types may have been feasible. Thus, this descriptive study is mainly focused on the impact of different meal compositions that naturally occur from habitual intake of the different diet types. In order to match protein composition across diets, hemp protein was added to the vegan regimen. While a strict vegetarian diet has been shown to improve risk factors associated with metabolic diseases; it is unknown whether a high calorie high protein vegan dinner would have produced a similar fasting metabolic signature as the animal diet previously published [9, 29]. Additional research is needed to understand if a 2-day meal plan with a high

protein and BCAA vegan dinner, matched to the animal dinner composition, would produce the same fasting metabolic signature or if a lower protein animal dinner would produce a more optimal metabolic signature.

Liberalization of the animal and vegan meal plans used in our research could improve the metabolic impact of the meals and snacks. The Nordic diet is characterized by a high content of fruits and vegetables, plants from the countryside, whole grains, nuts, seafood, free-range livestock and game [34].The macronutrient composition of the animal and vegan diets and TDF content of the vegan diet match the Nordic diet, which could represent the liberalization of our animal menu plan and which has been shown to reduce diabetes risk [13]. The flexitarian or semi-vegetarian diet represents an alternative approach to the liberalization of the animal diet that also emphasizes plant foods with only periodic animal protein intake [16]. See Diet Personalization Supplement.

CONCLUSIONS

We expected to observe a sub-optimal metabolic postprandial profile from the animal meals that would reflect our previous fasting findings in this same cohort. However, the high BCAA protein supplemented vegan breakfast produced the same elevated BCAA-associated metabolic signature as the animal diet produced from our fasting results, reflecting the lower BCAA protein load in the animal breakfast. Our postprandial analysis demonstrated glucose, insulin, AA, TG, bile acid and fatty acid plasma biomarkers varied with diet nutrient composition irrespective of diet type; and that fasting analysis alone is not sufficient to diagnose diet impact. Additionally, our results suggest BCAA content, irrespective of diet type and protein source, may have a negative impact on metabolic health. Liberalization of both diet strategies to optimize metabolic signatures should be tested in future research.

ACKNOWLEDGEMENTS

We would like to thank Cristiana Milone for her work generating the menus and diet analyses; the Metabolic team at the Clinical Development Unit for their role in running the study at the metabolic unit (MU) and Irina Monnard at NRC; Richard Cote (NIHS) for support in nutritional data integration and Frederic Ronga (NIHS) for his assistance on the diet intervention analysis. Jorg Hager and Jim Kaput (NIHS) for scientific discussions. Jan van der Greef for his scientific review and discussions; Eugenia Migliavacca for her statistical insights.

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GENDER DIMORPHISM SUPPLEMENT

Most cellular and human clinical research has been performed either on males only or both males and females, controlling for sex and gender differences to homogenize the data as opposed to differentiating between sex or gender specific responses. We feel it is important to acknowledge the gender and sex differences observed in our research in order to expose potential hypotheses for future testing, the results of which can be used to strengthen clinical practice. This is necessary to improve the healthcare of both genders.

Gender dimorphic postprandial response
Breakfast AUC results for plasma Insulin, TGs, PRO, and ALA were significantly lower for women whose lower calorie and nutrient needs necessitated lower intakes from CHO, fat and PROT. Lunch AUC results for plasma TGs were, also, significantly lower for women. Essential amino acids, BCAAs and 8 AAs were significantly higher for women at lunch which was not consistent with differences in intake (FDR<0.10). Five bile acids were elevated in women after breakfast and ten bile acids were higher for women after lunch ($p < 0.05$, FDR>0.10) (**Figure 2, Figure 4**,**Table S4, Table 1**) .

Gender dimorphic postprandial response leads to more insight on personalization
Triglyceride and insulin responses in women at breakfast and lunch were lower, likely reflecting lower nutrient intakes; however, differences in physiology and metabolism may have played a role. For example, a lower TG response may also relate to known increased postprandial skeletal muscle clearance of TG in women, which leads to lower plasma concentrations (**Figure 2,Table S4**) [1]. Estrogen regulates insulin sensitivity in females and may have further augmented the lower plasma insulin response to the diet interventions seen in the premenopausal women in our study (**Table S2**) [2, 3]. Additionally, previously published research suggests females may have been more responsive to the triglyceride and insulin lowering effects of higher soluble fiber in the vegan diet (**Figure S6**) [4].

Analysis of gender differences in dietary intake during the breakfast, lunch and dinner timeperiods revealed men had significantly higher nutrient intakes, as expected. This is due to higher calorie and nutrient needs of men as the composition of the meals provided were matched to age, height, weight and sex (**Table S4**). This was reflected in significantly greater plasma AUC AA concentrations in men at breakfast, however, women had higher plasma AUC AA at lunch (**Table S2**). Elevated plasma AA in women despite lower protein intake may reflect an accumulation of plasma AA over time due

to slower female mechanisms that regulate AA utilization and protein metabolism and a gender specific response to the prolonged nutrient digestion effects of TDF [4, 5].
The trend of elevated bile acids in women after breakfast and lunch despite lower dietary fat intake, may be linked to the known effect of sex hormones and increased expression of Cyp7a1, as well as a known differential bile acid response of females to high TDF intake (**Figure 4, Table S2, Table S3**) [4, 6-8]. Thus, consideration of differences in gender response is an important diagnostic component of personalized diet assessment and therapy.

CONCLUSION

Gender dimorphism in metabolic signatures was observed. Lower nutrient intakes in women led to lower TG and insulin responses. Lower insulin and elevated bile acid responses may have been further augmented by estrogen. Prolonged plasma amino acid elevation in women may have been influenced by a gender specific response to soluble TDF and gender specific differences in metabolic rate. Thus, consideration of differences in gender response is an important diagnostic component of personalized diet assessment and therapy.

DIET PRESCRIPTION FOR PERSONALIZATION

Measurement of postprandial response creates opportunities for diet personalization. The results from our study suggest liberalization of the animal and vegan diet plans used in our research could improve the metabolic impact of the meals and snacks. The following are examples of recommended modifications to both strategies to personalize the diet prescription based on diet type and postprandial response.

Modification of the vegan diet to vegetarian
A reduction in the muesli, cashew and hemp protein portions would reduce calories and nutrients at breakfast to moderate TG, insulin, glucose and AA response. Inclusion of cheese at lunch would increase plasma fatty acid concentrations. A decrease in the portion of rice and beans at lunch and removal of the Kombucha drink would reduce CHO and blood glucose.

Modification of the animal diet to include vegetarian components
An increase in TDF at breakfast with the addition of fruit, such as berries, may increase bile acid concentrations. The addition of TDF at lunch obtained by replacing chips with cooked vegetables, apple sauce with a fresh apple, as well as the hamburger roll with a whole wheat variety, could help prolong the absorption of amino acids from the

hamburger meal, reducing the amino acid spike observed after lunchtime. A reduction in the portion of chicken at dinner, replacement of standard pasta with a whole wheat variety and replacement of the apricot tart with fresh fruit and nuts would decrease the high amino acid and simple sugar intakes. Indeed, those may have contributed to the elevated AA, insulin and TG observed from the day 3 fasting results [9]. The addition of cottage to cheese to the afternoon snack would replace the protein intake removed from dinner.

CONCLUSION

The Nordic diet is characterized by a high content of fruits and vegetables, plants from the countryside, whole grains, nuts, seafood, free-range livestock and game [10]. The macronutrient composition of the animal and vegan diets and TDF content of the vegan diet match the Nordic diet, which could represent the liberalization of our animal menu plan and which has been shown to reduce diabetes risk [11]. The flexitarian or semi-vegetarian diet represents an alternative approach to the liberalization of the animal diet that also emphasizes plant foods with only periodic animal protein intake [12].

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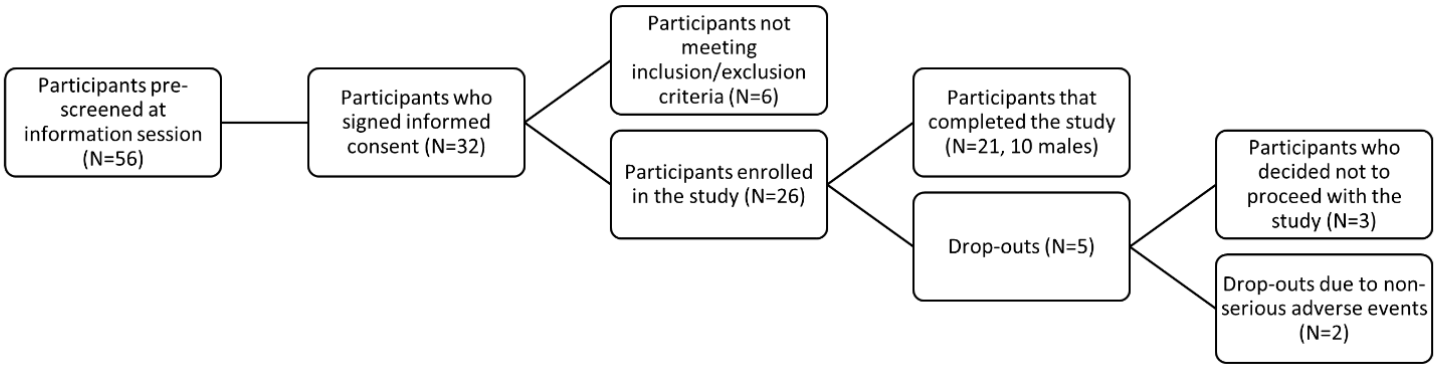
SUPPLEMENTARY FIGURES



Supplementary Figure S1. Study flow chart.

ANIMAL DIET Menu

Breakfast
Croissant
Strawberry jam
Coffee with milk and sugar

Snack
Yogurt Nature
Fresh apple

Lunch
Beef Hamburger with mustard and fresh tomato slices
Chips
Cucumber salad
Applesauce

Snack
Crackers

Dinner
Dijon chicken with pasta
Mixed lettuce with balsamic vinaigrette
Apricot tart

Drinks
Water

"Emergency Snack"
Yogurt, skimmed with fruit
10 Raw plain almonds

Kcals
1600
1800
2000
2200
2400
2600
2800
3000

VEGAN DIET Menu

Kcals
1600
1800
2000
2200
2400
2600
2800
3000

Breakfast
Healthy quinoa rice milk müesli with cashew butter
Hemp protein powder
Black coffee

Snack
Homemade hummus + Hemp protein powder
Carrots, cucumber
Gluten-free bread, Kombucha drink

Lunch
Brown rice and red beans salad with baby spinach, white balsamic vinaigrette
Gluten Free Bread
Green Tea

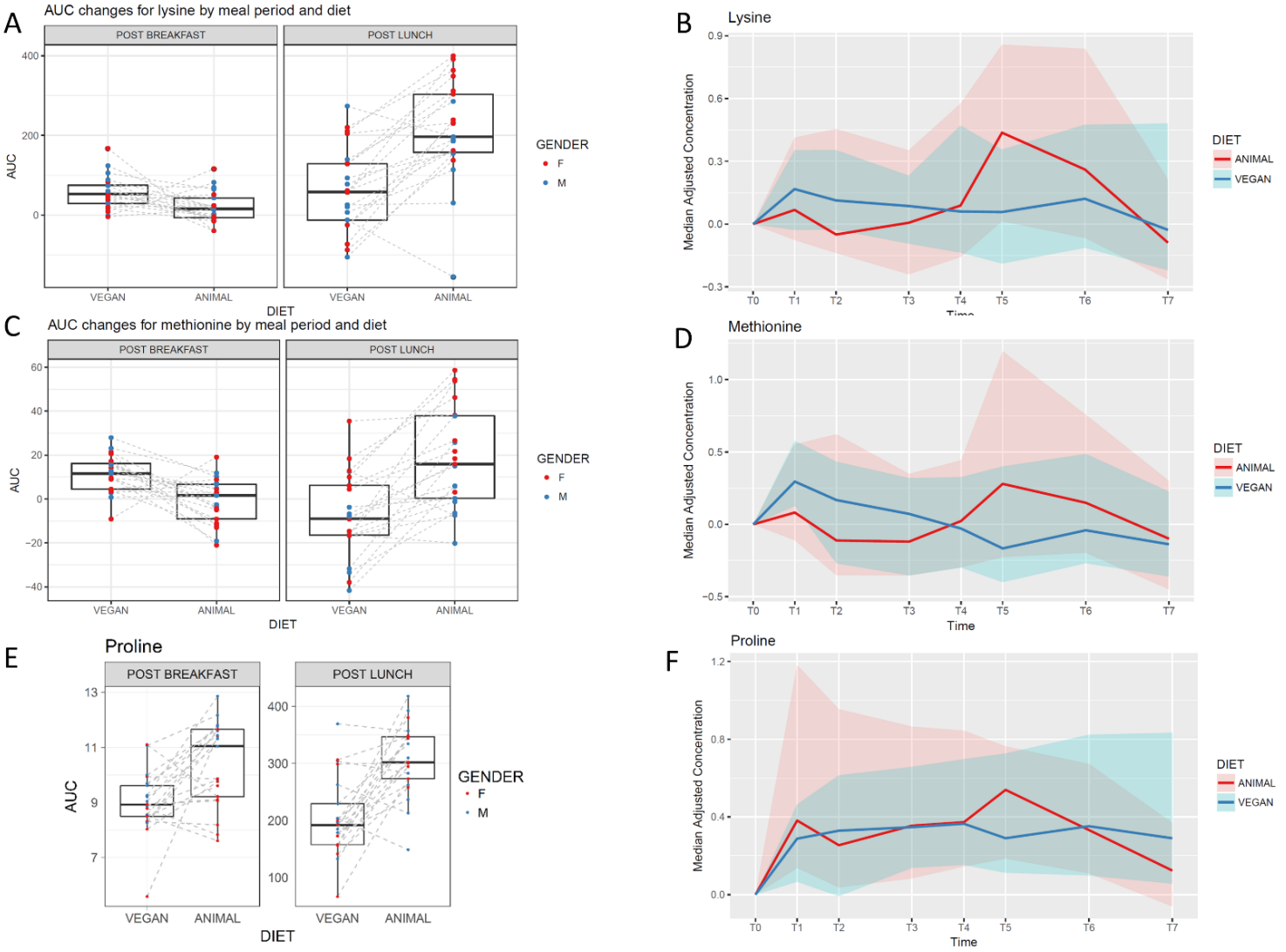
Snack
Fresh banana with soy yogurt

Dinner
Asian lentil coconut curry +Hemp protein powder
Gluten-free bread
Ginseng and green tea

Drinks
Water

"Emergency Snack"
Soy Yogurt Nature (Coop Naturaplan)

Supplementary Figure S2. The animal and vegan diet menu plans were the same each day for 2 days. Food portions were provided based on 8 personalized calorie plans in accordance with the caloric needs of the individual participants. Hemp protein powder was used for the vegan diet to boost total protein.


A
AUC changes for lysine by meal period and diet
POST BREAKFAST
POST LUNCH
AUC
DIET
VEGAN
ANIMAL
GENDER
F
M
B
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DIET
ANIMAL
VEGAN
T0
T1
T2
T3
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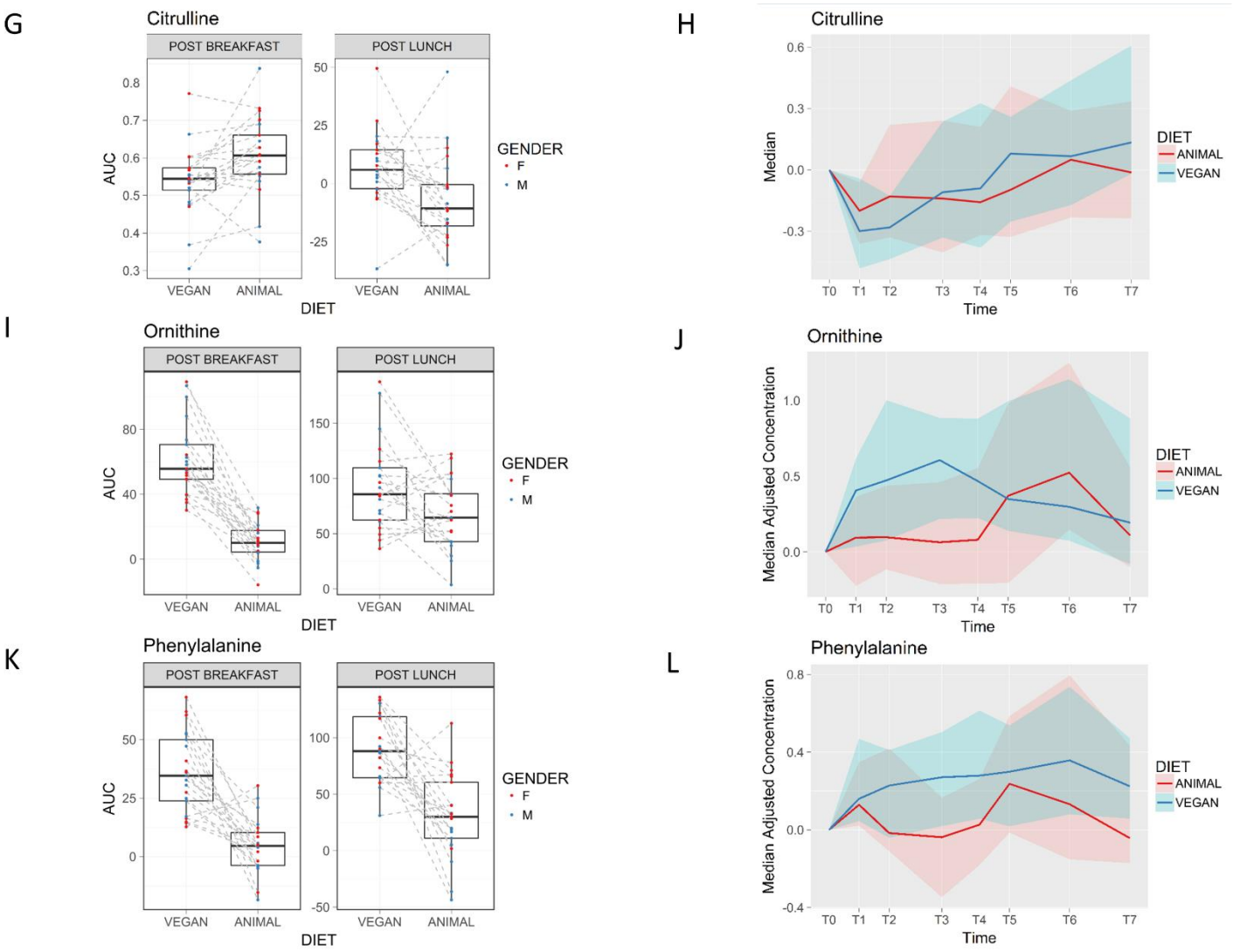
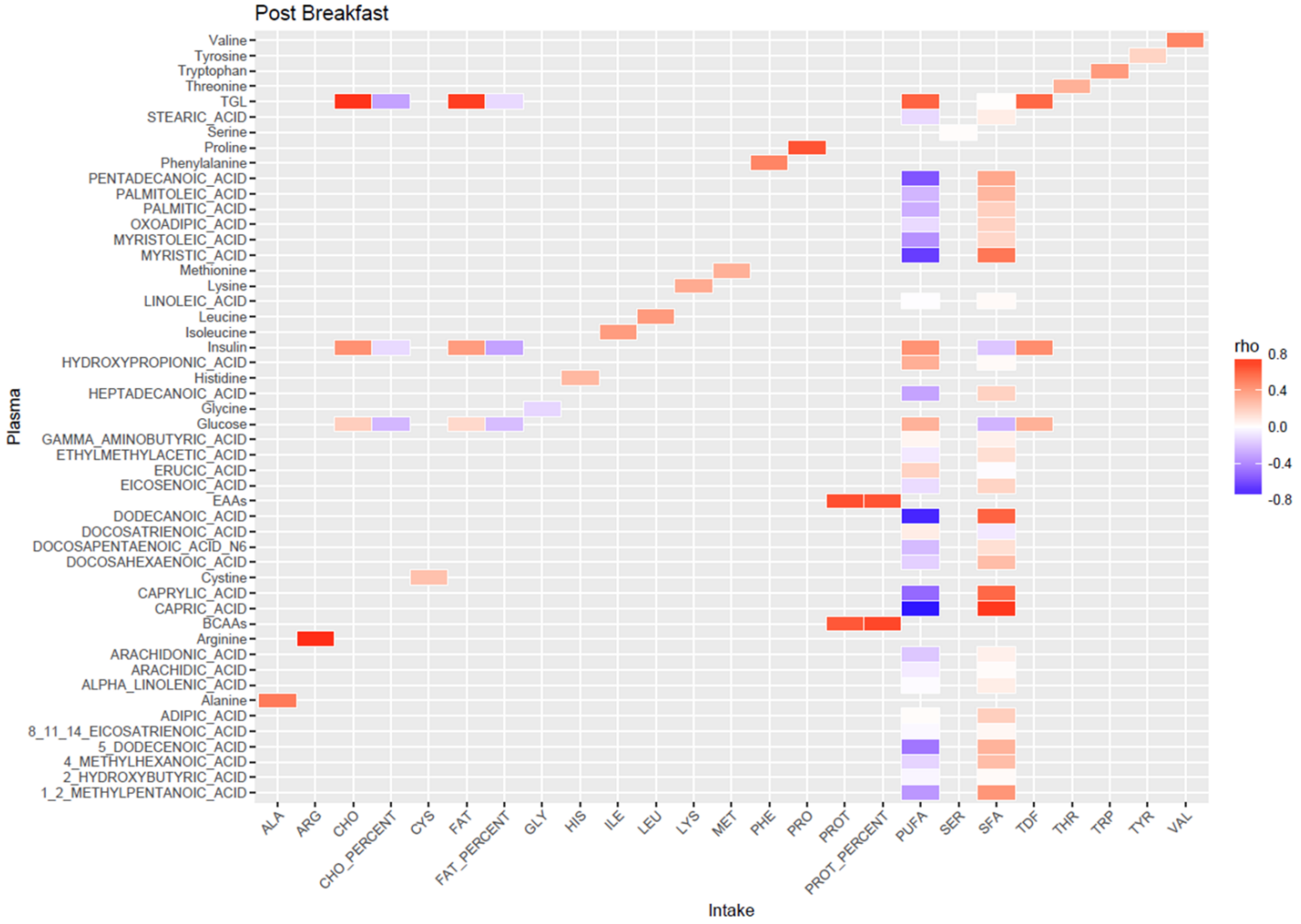
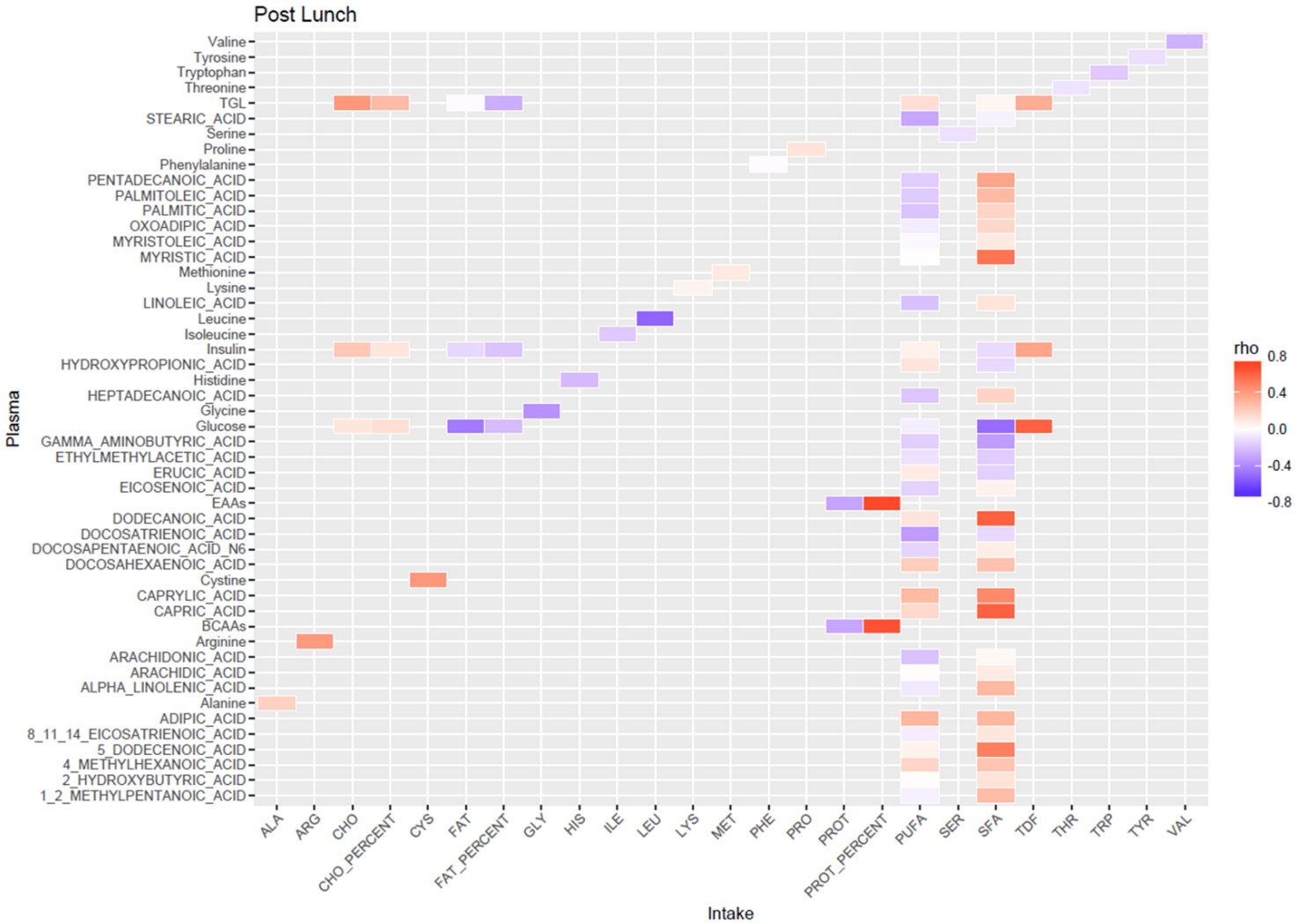


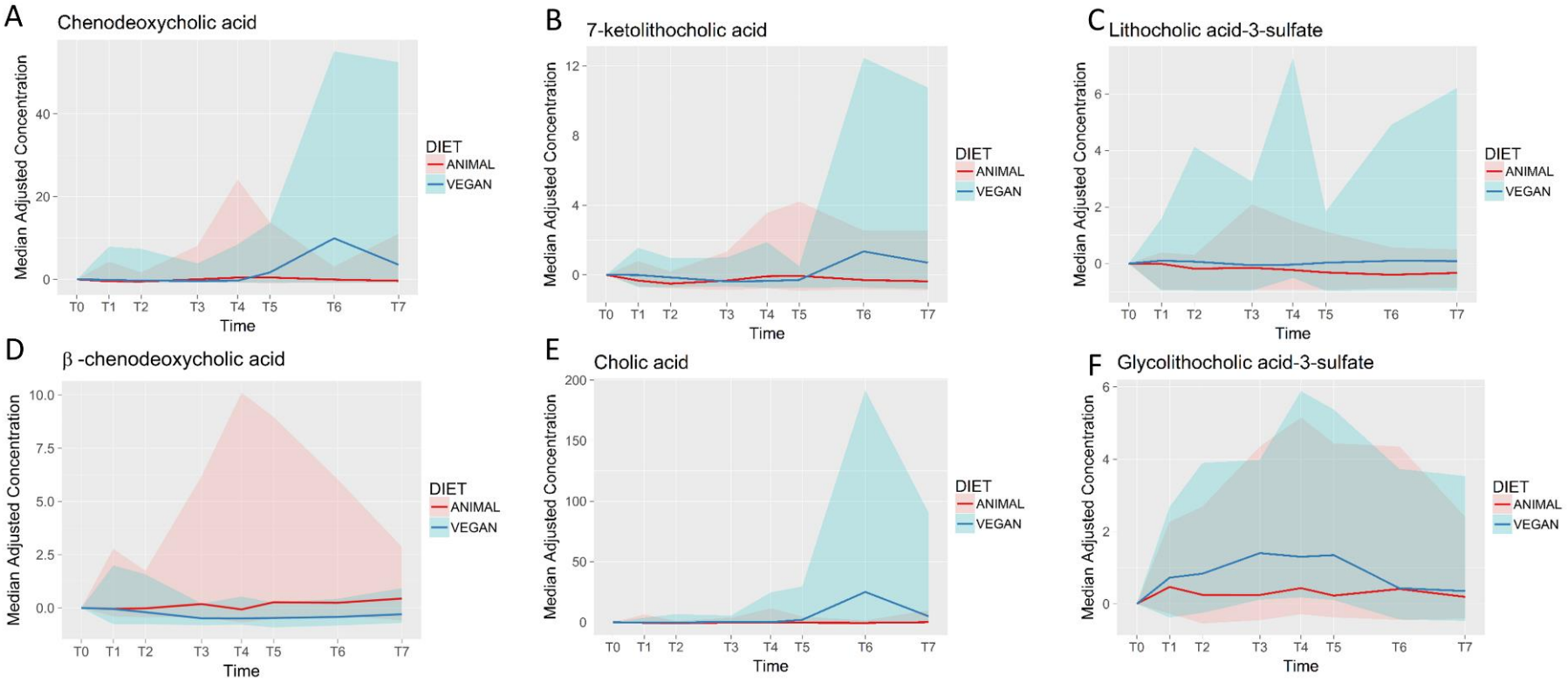
Supplementary Figure S3. (A-L) Amino acids with significant differences observed in AUC timeframes comparing vegan and animal breakfast, vegan and animal lunch, vegan vs animal changes from breakfast to lunch and from ANOVA timepoint analyses. Statistics can be found in Table S2. Metabolic signatures differ according to timing (AUC comparisons); and Table S3. Metabolic signatures differ according to timing (Timepoint comparisons).



Supplementary Figure S4. Spearman correlations between significant plasma metabolites and diet intake are graphically represented with a heat map for the post-breakfast AUC response. Statistics can be found in Table 2. Plasma biomarker x diet correlations.



Supplementary Figure S5. Spearman correlations between significant plasma metabolites and diet intake are graphically represented with a heat map for the post-lunch AUC response. Statistics can be found in Table 2. Plasma biomarker x diet correlations.



Supplementary Figure S6. (A-F) Line graphs of the medians of representative bile and fatty acids demonstrating statistically significant changes from the vegan versus animal diets throughout the postprandial timeperiods. Statistical results can be found in Table S3. Metabolic signatures differ according to timing (Timepoint comparisons).

SUPPLEMENTARY TABLES

Supplementary Table 1. Meal and snack comparisons

	Breakfast			Morning snack			Lunch			Afternoon snack			Dinner		
Nutrient	**Animal diet means**	**Vegan diet means**	***p*-value***	**Animal diet means**	**Vegan diet means**	***p*-value***	**Animal diet means**	**Vegan diet means**	***p*-value***	**Animal diet means**	**Vegan diet means**	***p*-value***	**Animal diet means**	**Vegan diet means**	***p*-value***
Kilocalories (Kcal)	405.81	596.37	**1.49E-04**	192.54	299.14	5.72E-03	569.50	344.60	**6.37E-05**	104.71	211.67	**4.04E-05**	851.65	351.56	**6.38E-05**
Protein (g)	8.90	22.40	**6.16E-05**	6.99	14.40	2.22E-03	24.98	14.72	**6.37E-05**	2.94	7.52	**4.73E-05**	59.62	18.58	**6.37E-05**
% Kcals from protein	8.41	15.14	**6.04E-05**	14.66	18.18	4.37E-03	17.68	17.51	6.51E-01	11.30	13.59	1.25E-03	28.14	20.20	**7.38E-05**
Alanine (mg)	350.38	834.63	**7.14E-05**	314.80	555.14	5.42E-03	1133.65	670.47	**6.36E-05**	96.54	333.83	**4.04E-05**	3142.47	735.26	**6.35E-05**
Arginine (mg)	436.33	1320.26	**6.24E-05**	253.52	1072.67	**1.31E-04**	1165.76	931.25	1.08E-03	105.38	569.83	**4.04E-05**	3470.18	1430.94	**6.35E-05**
Cysteine (mg)	152.33	285.62	**7.23E-05**	53.81	186.88	**1.50E-04**	374.88	152.95	**6.35E-05**	62.71	60.33	1.42E-01	957.74	210.80	**6.35E-05**
Glycine (mg)	267.16	679.39	**6.16E-05**	175.00	505.71	**1.51E-04**	1131.40	641.36	**6.36E-05**	119.36	308.58	**4.73E-05**	2904.32	680.99	**6.35E-05**
Histidine (mg)	222.43	418.63	**7.14E-05**	181.46	336.29	2.21E-03	661.62	386.48	**6.37E-05**	56.57	249.50	**2.95E-05**	1854.68	416.13	**6.35E-05**
Isoleucine (mg)	432.95	632.39	**2.22E-04**	429.73	627.26	4.75E-02	997.77	612.43	**6.37E-05**	112.52	319.67	**4.73E-05**	3163.58	720.35	**6.35E-05**
Leucine (mg)	849.90	1325.48	**1.46E-04**	732.69	896.43	1.86E-01	1702.81	1075.52	**6.37E-05**	218.38	98.33	**6.44E-05**	4837.73	1181.73	**6.35E-05**
Lysine (mg)	605.90	643.88	6.26E-01	558.23	728.74	1.54E-01	1301.32	894.65	**6.36E-05**	58.05	440.33	**3.45E-05**	4373.74	983.80	**6.35E-05**
Methionine (mg)	244.52	298.68	2.59E-02	177.94	177.21	9.72E-01	446.77	213.35	**6.35E-05**	45.00	86.50	**6.44E-05**	1519.57	208.36	**6.35E-05**
Phenylalanine (mg)	468.10	742.58	**1.46E-04**	376.76	594.93	2.38E-02	907.87	732.30	2.21E-03	141.43	324.00	**5.52E-05**	2762.67	818.12	**6.35E-05**
Proline (mg)	936.02	915.70	9.72E-01	811.58	538.61	2.49E-03	15042.56	577.58	**6.36E-05**	364.77	48.67	**4.73E-05**	4060.44	710.22	**6.35E-05**
Serine (mg)	534.38	796.54	**1.46E-04**	432.36	638.58	4.75E-02	1029.08	769.57	**3.41E-04**	151.17	405.83	**4.73E-05**	1785.19	858.44	**6.30E-05**
Threonine (mg)	380.38	612.80	**1.46E-04**	305.60	512.14	1.23E-02	822.52	573.57	**9.85E-05**	84.67	293.00	**4.04E-05**	2584.56	680.11	**6.35E-05**
Tryptophan (mg)	112.43	156.23	7.26E-04	80.74	111.31	7.62E-02	301.45	159.54	**1.50E-04**	33.95	95.50	**5.52E-05**	863.38	149.21	**6.35E-05**
Tyrosine (mg)	392.10	492.65	2.82E-02	354.14	448.12	1.64E-01	735.05	399.39	**6.35E-05**	87.33	251.00	**4.73E-05**	1942.92	570.93	**6.35E-05**
Valine (mg)	528.90	834.54	**1.46E-04**	536.50	602.29	5.31E-01	1094.67	714.06	**6.35E-05**	126.81	342.00	**4.04E-05**	3115.46	883.45	**6.35E-05**
Carbohydrate (g)	45.96	62.93	**2.52E-04**	22.61	34.71	2.19E-03	52.69	46.83	**6.36E-05**	20.62	35.00	**3.45E-05**	111.36	41.98	**6.37E-05**
% Kcals from carbohydrate	45.83	42.40	**1.43E-04**	46.57	50.06	5.78E-01	37.08	52.82	1.14E-01	79.18	67.65	1.45E-03	51.99	48.98	7.62E-02
Total fiber (g)	2.01	7.41	**6.14E-05**	2.63	7.73	**1.72E-04**	5.86	10.47	**6.36E-05**	0.94	4.25	**2.95E-05**	5.37	7.60	2.21E-03
Total fat (g)	19.67	27.52	**1.92E-04**	7.64	9.38	2.81E-01	27.96	10.46	**6.35E-05**	1.19	4.17	1.03E-03	15.38	11.51	**2.23E-04**
% Kcal from fat	43.45	41.30	**1.90E-04**	35.99	25.53	**9.90E-05**	44.02	28.89	**6.36E-05**	9.73	16.55	1.68E-03	16.31	29.48	**6.38E-05**
Polyunsaturated fat (g)	0.83	7.21	**6.16E-05**	0.19	6.00	**7.34E-05**	8.73	6.02	3.90E-03	0.43	2.10	8.31E-04	4.69	6.53	3.90E-03
Monounsaturated fats (g)	3.67	14.77	**6.16E-05**	2.31	1.21	**5.63E-04**	11.58	3.43	**6.35E-05**	0.26	0.92	**5.62E-05**	5.86	1.55	**6.13E-05**
Saturated fat (g)	14.37	5.27	**6.16E-05**	3.95	1.54	**8.47E-05**	7.44	1.37	**6.37E-05**	0.40	0.77	8.31E-04	3.38	2.39	1.11E-02

***p values bolded for q<0.10**

Supplementary Table S2. Metabolic signatures differ according to AUC comparisons

	AUC Post-Breakfast			AUC Post-Lunch		
Marker	**Gender *P* value***	**Diet effect***	**Diet *P* value***	**Gender *P* value***	**Diet effect***	**Diet *P* value***
Amino acids (nmol/ml)						
Phenylalanine	8.99E-01	31.32	**1.43E-06**	**1.16E-02**	60.06	**4.81E-06**
Lysine	2.52E-01	36.78	**9.06E-03**	**4.02E-02**	-145.93	**2.24E-05**
Methionine	1.75E-01	12.40	**4.56E-04**	**9.01E-03**	-26.35	**3.96E-05**
Proline	**7.85E-04**	-1.44	**6.55E-04**	7.28E-01	-102.95	**5.04E-05**
Valine	6.32E-01	120.44	**3.59E-05**	**2.69E-03**	147.27	**2.99E-04**
Arginine	1.77E-01	4.76	**1.29E-10**	9.87E-01	0.14	**4.10E-03**
Citrulline	8.27E-02	-0.07	**7.56E-03**	7.23E-01	13.94	**1.62E-02**
Ornithine	1.52E-01	50.89	**2.58E-09**	6.70E-01	24.92	**2.27E-02**
Threonine	9.11E-01	31.84	**1.99E-02**	**1.66E-02**	33.94	1.80E-01
BCAAs	5.30E-01	209.32	**9.08E-04**	**3.88E-03**	91.70	1.81E-01
Leucine	3.78E-01	38.96	**1.24E-02**	**6.21E-03**	-27.68	2.21E-01
Acetyltryptophan				2.88E-02	-0.42	2.52E-01
Tyrosine				**1.62E-03**	11.23	3.22E-01
Glycine				**3.40E-02**	27.82	6.84E-01
EAAs	4.06E-01	326.33	**5.14E-05**	**7.10E-04**	7.78	9.48E-01
Asparagine	9.92E-01	0.10	**7.01E-06**			
Tryptophan	9.28E-01	24.70	**4.64E-05**			
Isoleucine	4.18E-01	26.77	**1.34E-02**			
Histidine	9.93E-01	13.17	**2.80E-02**			
Alanine	**6.57E-03**	47.09	1.96E-01			
Fatty acids (ng/ml)						
Dodecenoic acid (C12:1)	1.34E-01	-2489.25	**2.94E-06**	1.11E-01	-0.23	**1.38E-06**
Capric acid (C10:0)	7.95E-01	-0.39	**1.27E-09**	9.00E-01	-0.35	**1.05E-05**
Myristic acid (C14:0)	2.06E-01	-5854.76	**2.22E-03**	3.09E-01	-0.13	**5.14E-05**
5-Dodecenoic acid (C12:1)	2.57E-01	-2626.74	**3.36E-03**	3.22E-01	0.00	**2.97E-04**
Myristoleic acid (C14:1)				1.03E-01	-881.28	**1.86E-03**
Pentadecanoic acid (C15:0)	2.28E-01	-0.10	**3.86E-05**	2.90E-02	-0.14	**6.96E-03**
Eicosenoic acid (C20:1)				3.04E-02	-1035.60	3.69E-01
1,2-Methylpentanoic acid	8.01E-01	-0.45	**6.77E-03**			
Caprylic acid (C8:0)	9.22E-01	-1872.33	**2.94E-04**			
Heptadecanoic acid (C17:1)	9.33E-01	-0.87	2.84E-02			
Docosahexaenoic acid (C22:6)	4.36E-01	-3886.31	4.15E-02			
Clinical variables						
Insulin (µU/mL)	**4.62E-02**	0.38	**3.27E-03**	3.98E-01	-0.45	**5.11E-02**
Glucose (mM/L)	8.75E-01	0.35	**1.29E-02**	7.15E-01	0.16	**5.60E-02**
TGL (mM/L)	**3.96E-04**	0.72	**8.70E-03**	**6.90E-03**	-0.31	3.75E-01
Metabolites						
Norleucine				2.65E-01	-10.55	**5.45E-03**
3-methyl-2-oxovaleric acid	3.87E-01	-0.20	**7.73E-04**	3.67E-01	-0.03	2.87E-02
Pimelic acid				1.56E-01	-1.96	3.62E-02
Citric acid	8.62E-01	-7.47	2.96E-02	5.01E-01	-1.13	4.15E-02
Oxoadipic acid				1.88E-01	-0.31	2.03E-02
Bile acids (nmol/L)						
b-Chenodeoxycholic acid (bCDCA)	4.82E-01	-1.04	**1.84E-02**	3.64E-02	-1.66	**2.82E-05**
Cholic acid (CA)	1.09E-01	1.64	**1.60E-02**	7.31E-01	2.90	**2.91E-05**
Hyocholic acid (HCA)	5.66E-02	1.88	3.01E-02	5.52E-02	3.08	**7.62E-05**
b-dehydrochenodeoxycholic acid (bDHCDCA)				2.79E-02	-1.13	**1.41E-03**
Lithocholic acid-3-sulfate (LCA-3S)	2.40E-01	1.85	**8.88E-03**	6.05E-01	2.14	**1.87E-03**
7-Dihydrocholestanoic acid (7-DHCA)	3.73E-02	0.36	7.62E-02	4.18E-03	1.08	**2.42E-03**
Glycolithocholic acid-3-sulfate (GLCA-3S)	7.94E-01	2.57	**1.98E-03**	4.39E-01	2.99	**5.59E-03**
Chenodeoxycholic acid (CDCA)				3.68E-01	1.45	**6.01E-03**
Ursodeoxycholic acid (bUDCA)				1.52E-01	-0.99	**7.53E-03**
Nor deoxycholic acid (NorDCA)				9.09E-02	0.65	**1.78E-02**
Deoxycholic acid (DCA)	2.52E-02	0.35	3.14E-01	1.24E-01	410.43	**1.96E-02**
7-Ketolithocholic acid (7-ketoLCA)	6.37E-02	0.57	**1.22E-02**	1.26E-01	0.91	**1.97E-02**
b-Deoxycholic acid (bDCA)				8.47E-03	-0.27	**2.09E-02**
Ursodeoxycholic acid (UDCA)	3.93E-02	0.31	2.97E-01	4.41E-02	0.78	7.42E-02
Taurohyocholic acid (THCA)				4.99E-02	0.15	6.19E-01
Hyodeoxycholic acid (HDCA)				7.68E-03	0.21	8.17E-01
Taurodeoxycholic acid (TDCA)	4.75E-02	0.45	4.36E-01	3.32E-02	-0.02	9.34E-01
Tauorlithocholic acid (TLCA)	7.65E-01	1.02	**6.39E-04**			
Isoithocholic acid (IsoLCA)	4.63E-02	-1.01	**7.13E-03**			
7-Ketolithocholic acid (7-ketoLCA)	6.37E-02	0.57	**1.22E-02**			
Gycolithocholic acid (GLCA)	5.01E-02	1.35	**1.46E-02**			

AUC, Area Under the Curve. *Bolded for q<0.10. **Vegan vs. animal. Blank cells signify no significant gender or diet differences.

Supplementary Table S3. Metabolic signatures differ according to timing (Timepoint comparisons)

Marker	T0	T1	T2	T3	T4	T5	T6	T7
Amino acids (nmol/ml)								
Alanine	**1.00E+00**	9.80E-01	**1.53E-04**	3.60E-01	**1.17E-03**	**1.72E-03**	1.54E-01	**8.74E-09**
Arginine	**1.00E+00**	**9.33E-15**	**0.00E+00**	**0.00E+00**	**4.07E-07**	**3.40E-02**	5.32E-01	**2.08E-08**
Asparagine	**1.00E+00**	**6.90E-03**	**5.48E-10**	**7.11E-08**	1.24E-01	4.26E-01	**5.56E-03**	**2.69E-08**
BCAAs	**1.00E+00**	1.79E-01	**3.15E-07**	**5.10E-07**	**4.60E-07**	3.96E-01	7.96E-01	**4.81E-04**
Citrulline	**1.00E+00**	**6.90E-03**	**8.36E-06**	3.89E-01	3.13E-01	**2.06E-04**	3.83E-01	**2.29E-06**
Cystine	**1.00E+00**	2.06E-01	5.37E-02	2.99E-01	5.20E-01	**2.08E-03**	1.31E-01	1.02E-01
EAAs	**1.00E+00**	1.70E-01	**7.40E-08**	**1.49E-06**	**5.27E-04**	**5.50E-03**	5.83E-01	**4.44E-06**
Glutamine	**1.00E+00**	6.97E-01	5.37E-02	1.27E-01	**3.76E-02**	5.17E-01	1.35E-01	**1.41E-04**
Histidine	**1.00E+00**	9.22E-01	**6.14E-04**	**4.91E-02**	3.09E-01	**4.01E-07**	6.14E-01	**2.08E-08**
Isoleucine	**1.00E+00**	9.43E-01	**2.96E-04**	**4.06E-04**	**3.44E-03**	**3.90E-03**	7.99E-02	2.61E-01
Leucine	**1.00E+00**	9.80E-01	**8.71E-05**	**7.39E-04**	**2.90E-03**	**4.67E-03**	2.72E-01	**9.00E-02**
Lysine	**1.00E+00**	5.23E-02	**8.71E-05**	3.09E-01	7.18E-01	**1.09E-08**	**1.45E-03**	**2.59E-02**
Methionine	**1.00E+00**	**1.49E-02**	**1.18E-05**	**2.41E-03**	6.49E-01	**1.43E-12**	**2.30E-05**	7.36E-01
Ornithine	**1.00E+00**	**2.41E-10**	**9.56E-14**	**0.00E+00**	**0.00E+00**	5.17E-01	**1.45E-03**	**6.77E-03**
Phenylalanine	**1.00E+00**	1.70E-01	**7.40E-08**	**0.00E+00**	**7.26E-11**	1.10E-01	**7.05E-05**	**4.65E-10**
Proline	**1.00E+00**	**1.49E-02**	1.02E-01	9.67E-01	3.13E-01	**1.54E-03**	7.30E-01	**1.21E-08**
Serine	**1.00E+00**	4.28E-01	1.35E-01	2.24E-01	7.73E-01	**3.40E-02**	7.99E-02	**2.29E-06**
Threonine	**1.00E+00**	9.22E-01	**1.15E-04**	**2.32E-02**	2.16E-01	1.27E-01	3.27E-01	**2.47E-06**
Tryptophan	**1.00E+00**	**9.24E-03**	**1.77E-10**	**5.75E-08**	**5.27E-04**	**5.50E-03**	1.39E-01	**6.88E-07**
Tyrosine	**1.00E+00**	9.92E-01	**3.48E-03**	**2.31E-03**	**2.08E-02**	4.71E-01	8.18E-01	**1.54E-05**
Valine	**1.00E+00**	**5.54E-03**	**1.77E-10**	**2.66E-11**	**8.25E-13**	**3.43E-02**	1.54E-01	**2.52E-08**
Fatty acids (ng/ml)								
5-Dodecanoic acid (C12:1)	**1.00E+00**	6.31E-01	**1.68E-03**	**2.04E-08**	**1.03E-04**	**1.69E-02**	**3.05E-02**	**1.16E-02**
Capric acid (C10:0)	**1.00E+00**	**0.00E+00**	**0.00E+00**	**0.00E+00**	**0.00E+00**	**6.84E-14**	**6.47E-03**	**5.38E-06**
Caprylic acid (C8:0)	**1.00E+00**	**4.32E-05**	**1.50E-04**	**4.40E-06**	**1.03E-04**	**3.32E-02**	5.29E-01	4.52E-01
Dodecanoic acid (C12:0)	**1.00E+00**	4.50E-01	**4.75E-07**	**0.00E+00**	**6.89E-10**	**1.60E-02**	**2.09E-02**	**3.32E-03**
Myristic acid (C14:0)	**1.00E+00**	**1.19E-02**	**5.05E-08**	**0.00E+00**	**6.89E-10**	**9.42E-05**	**1.79E-02**	**2.64E-06**
Myristoleic acid (C14:1)	**1.00E+00**	6.79E-01	**4.39E-03**	**1.02E-03**	**2.33E-05**	**3.10E-04**	**6.47E-03**	**1.91E-02**
Palmitoleic acid (C16:1)	**1.00E+00**	**1.29E-02**	**1.68E-03**	**1.41E-04**	**4.31E-02**	6.59E-01	7.34E-01	**1.16E-02**
Pentadecanoic acid (C15:0)	**1.00E+00**	**5.72E-05**	**1.11E-03**	**1.03E-08**	**1.44E-03**	**3.35E-02**	2.25E-01	**1.98E-04**
Clinical variables								
Insulin (µU/mL)	**1.00E+00**	7.18E-01	**5.06E-02**	**3.52E-05**	3.82E-01	3.52E-01	8.20E-02	4.92E-01
Glucose (mM/L)	**1.00E+00**	1.78E-01	**1.83E-03**	**1.51E-07**	**4.39E-04**	**4.44E-04**	**2.73E-03**	**3.43E-02**
TGL (mM/L)	**1.00E+00**	**5.92E-07**	**1.01E-07**	4.91E-01	2.91E-01	9.57E-01	7.15E-01	**1.31E-02**
Metabolites								
Citric acid	**1.00E+00**	**4.77E-02**	1.40E-01	**7.46E-03**	**9.88E-05**	**2.91E-04**	9.94E-01	**1.16E-02**
Methylsuccinic acid	**1.00E+00**	6.31E-01	6.68E-01	**7.46E-03**	**5.02E-04**	1.63E-01	**3.05E-02**	9.01E-01
Succinic acid	**1.00E+00**	6.18E-01	9.91E-01	**9.71E-02**	**5.15E-05**	**3.35E-02**	**4.67E-02**	8.84E-01
Bile acids (nmol/L)								
12-ketoLCA_hawaii	**1.00E+00**	2.28E-01	4.01E-01	6.84E-01	**8.15E-03**	**4.70E-02**	**7.83E-06**	2.14E-01
3-Dihydrocholestanoic acid (3-DHCA)	**1.00E+00**	**1.30E-02**	**8.79E-02**	6.84E-01	7.85E-01	8.58E-01	**1.57E-04**	**1.98E-02**
7-Dihydrocholestanoic acid (7_DHCA)	**1.00E+00**	2.88E-01	3.91E-01	7.20E-01	7.85E-01	2.18E-01	**5.54E-06**	**2.98E-03**
7-Ketolithocholic acid (7-ketoLCA)	**1.00E+00**	**4.91E-02**	**2.71E-02**	9.11E-01	1.75E-01	2.46E-01	**6.06E-06**	**9.00E-09**
b-Chenodeoxycholic acid (bCDCA)	**1.00E+00**	7.16E-01	**9.99E-02**	**6.01E-07**	**1.93E-05**	**4.47E-10**	**1.13E-09**	**1.30E-05**
b-Deoxycholic acid (bDCA)	**1.00E+00**	9.02E-01	1.03E-01	**3.71E-04**	**7.60E-03**	**2.50E-05**	**6.02E-02**	4.56E-01
b-Dehydrochenodeoxycholic acid (bDHCDCA)	**1.00E+00**	**2.98E-02**	6.28E-01	**1.57E-02**	**1.93E-05**	**3.67E-09**	4.75E-01	**9.79E-02**
b-Ursodeoxycholic acid (bUDCA)	**1.00E+00**	8.13E-01	5.73E-01	1.31E-01	1.31E-01	**3.85E-03**	**2.55E-04**	**3.53E-05**
Cholic acid (CA)	**1.00E+00**	5.72E-02	**6.77E-02**	**7.22E-02**	4.41E-01	**2.50E-05**	**0.00E+00**	**1.46E-07**
Chenodeoxycholic acid (CDCA)	**1.00E+00**	**4.91E-02**	1.33E-01	1.18E-01	**4.07E-02**	4.11E-01	**2.94E-06**	**2.14E-06**
Chenodeoxycholic acid-3-glycine (CDCA-3G)	**1.00E+00**	8.63E-01	5.38E-01	1.38E-01	**2.33E-02**	**6.50E-03**	6.41E-01	2.20E-01
Deoxycholic acid (DCA)	**1.00E+00**	**4.91E-02**	3.81E-01	1.31E-01	6.34E-02	9.23E-01	**2.98E-06**	**2.93E-06**
Dehydrolithocholic acid (dehydroLCA)	**1.00E+00**	**4.23E-05**	**3.95E-02**	7.35E-01	8.38E-01	9.23E-01	9.59E-01	8.40E-01
GCA_hawaii	**1.00E+00**	**5.95E-09**	**2.61E-02**	2.88E-01	5.06E-01	4.24E-01	**2.25E-02**	1.13E-01
GHCA_hawaii	**1.00E+00**	**8.42E-05**	1.33E-01	1.31E-01	2.79E-01	**6.47E-03**	9.29E-01	7.85E-01
Glycolithocholic acid-3-sulfate (GLCA-3S)	**1.00E+00**	1.74E-01	**8.34E-04**	**8.71E-04**	**1.93E-05**	**1.56E-06**	6.64E-01	2.38E-01
Hyocholic acid (HCA)	**1.00E+00**	4.01E-01	1.02E-01	3.67E-01	8.38E-01	1.45E-01	**6.93E-11**	**3.48E-09**
Hyodeoxycholic acid (HDCA)	**1.00E+00**	1.63E-01	5.73E-01	3.85E-01	1.31E-01	3.16E-01	**6.22E-02**	**5.45E-02**
Isoithocholic acid (IsoLCA)	**1.00E+00**	6.66E-01	**2.61E-02**	**5.42E-05**	**1.22E-02**	1.04E-01	4.37E-01	9.34E-01
Lithocholic acid (LCA)	**1.00E+00**	**4.91E-02**	3.94E-01	7.35E-01	**3.36E-02**	**9.44E-02**	9.59E-01	8.71E-01
Lithocholic acid-3-sulfate (LCA-3S)	**1.00E+00**	**4.91E-02**	**8.97E-03**	2.76E-01	**2.33E-02**	**3.02E-02**	**6.73E-06**	**4.06E-06**
Taurocholic acid (TCA)	**1.00E+00**	**3.20E-09**	**2.61E-02**	6.46E-01	8.27E-01	9.23E-01	**1.24E-02**	1.42E-01
Taurochenodeoxycholic acid (TCDCA)	**1.00E+00**	**4.37E-07**	**2.61E-02**	3.79E-01	8.38E-01	8.58E-01	**2.25E-02**	1.91E-01
Taurodeoxycholic acid (TDCA)	**1.00E+00**	**1.48E-08**	**8.97E-03**	1.25E-01	1.59E-01	9.23E-01	**7.87E-03**	**5.61E-02**
Taurohyocholic acid (THCA)	**1.00E+00**	**3.81E-04**	**8.79E-02**	5.29E-01	9.78E-01	1.45E-01	2.57E-01	7.66E-01
Tauorlithocholic acid (TLCA)	**1.00E+00**	**1.98E-04**	**1.02E-02**	3.67E-01	8.38E-01	9.23E-01	3.14E-01	8.99E-01
Ursodeoxycholic acid (UDCA)	**1.00E+00**	**5.17E-02**	1.68E-01	3.67E-01	1.31E-01	6.73E-01	**6.19E-05**	**1.54E-05**

Comparison is vegan verses animal. Anova analysis. False discovery rate adjusted values shown. Bolded for q<0.10.

Supplementary Table S4. Diet intake analysis by gender for breakfast, lunch and dinner timeperiods

	Mean Intake						Adjusted *p* value Female vs. Male*		
	Breakfast		Lunch		Dinner		Breakfast	Lunch	Dinner
Diet variable	Male	Female	Male	Female	Male	Female			
Alanine (mg)	2582.71	1575.18	2580.63	1919.80	5151.85	3268.98	**4.43E-03**	**4.35E-03**	**3.32E-03**
Arginine (mg)	3857.60	2378.41	3191.13	2391.41	6727.73	4107.84	**1.32E-02**	**2.18E-02**	**3.32E-03**
Total carbohydrate(g)	203.68	132.16	182.53	130.25	200.15	135.41	**1.07E-03**	**7.58E-03**	**4.43E-03**
Cysteine (mg)	846.90	525.68	736.75	572.82	1542.50	950.93	**5.86E-03**	**3.27E-03**	**3.32E-03**
Total fat (g)	82.00	48.05	48.88	39.16	36.88	24.50	**3.32E-03**	5.90E-01	**2.78E-02**
Glycine (mg)	2076.56	1218.81	2525.81	1905.16	4732.78	3017.12	**5.86E-03**	**4.35E-03**	**3.32E-03**
Histidine (mg)	1482.20	864.82	1526.78	1197.25	3028.40	1912.34	**4.41E-03**	**1.30E-02**	**3.32E-03**
Isoleucine (mg)	2722.85	1576.41	2308.23	1800.73	5234.50	3312.39	**4.43E-03**	**2.18E-02**	**3.32E-03**
Kilocalories (Kcal)	1874.80	1147.55	1421.20	1057.09	1608.60	1048.73	**1.53E-04**	**2.18E-02**	**4.43E-03**
Leucine (mg)	4834.95	2867.73	3561.60	2670.91	7986.05	4880.70	**3.34E-03**	**7.58E-03**	**3.32E-03**
Lysine (mg)	3314.45	1829.75	3102.70	2323.14	7112.85	4556.45	**3.34E-03**	**7.58E-03**	**3.32E-03**
Methionine (mg)	1141.75	677.09	911.45	682.68	2274.93	1440.02	**3.32E-03**	**4.35E-03**	**3.32E-03**
Monounsaturated fat (g)	27.04	17.35	19.17	13.47	10.44	7.53	**7.55E-03**	**3.23E-03**	1.79E-01
Phenylalanine (mg)	2787.85	1631.93	2437.10	1804.23	4839.40	3028.70	**3.34E-03**	**9.94E-03**	**3.32E-03**
Proline (mg)	4028.80	2450.20	18090.64	14163.50	6371.41	3926.45	**3.34E-03**	**7.58E-03**	**3.32E-03**
Protein (mg)	66.76	39.92	57.37	43.60	106.97	64.88	**4.43E-03**	**1.30E-02**	**3.34E-03**
Polyunsaturated fat (g)	18.97	9.91	19.17	15.57	16.16	9.14	**4.41E-03**	1.00E+00	**1.71E-02**
Serine (mg)	3071.08	1793.46	2703.41	2039.51	3718.66	2300.33	**3.34E-03**	**1.30E-02**	**3.32E-03**
Saturated fat (g)	32.89	18.07	11.23	8.85	7.40	5.02	**3.32E-03**	**1.30E-02**	**3.61E-02**
Total fiber (g)	24.95	15.10	25.11	18.26	19.42	10.68	**7.58E-03**	**3.59E-02**	**5.86E-03**
Threonine (mg)	2320.15	1347.98	2038.25	1533.32	4377.88	2734.48	**4.43E-03**	**1.69E-02**	**3.32E-03**
Tryptophan (mg)	590.75	342.48	664.35	523.25	1311.38	867.30	**3.32E-03**	**2.18E-02**	**3.32E-03**
Tyrosine (mg)	2164.45	1252.98	1687.68	1277.41	3428.85	2149.18	**3.34E-03**	**7.58E-03**	**3.32E-03**
Valine (mg)	3169.80	1895.34	2624.40	1962.20	5428.55	3387.98	**3.34E-03**	**4.35E-03**	**3.32E-03**

Mean diet intakes analyzed by gender for breakfast, lunch and dinner. *Adjusted p values bolded for q<0.10.