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Pamela L. Bay

University of Vermont, pbay@uvm.edu

Shae Rowlandson

University of Vermont

Jana Kraft

University of Vermont, jana.kraft@uvm.edu

Mina Peshavaria

University of Vermont

Thomas Jetton

University of Vermont, tjetton@uvm.edu

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Plant-Derived Bioactive Lipids Impacts Glucose Homeostasis and Energy Metabolism in Mice

Pamela L Bay¹, Mina Peshavaria², Shae Rowlandson², Tom Jetton², Jana Kraft¹

¹Department of Animal Science, and ²Department of Medicine, University of Vermont, Burlington, Vermont 05405

ABSTRACT:

There is a crucial need to identify and test sustainable alternatives to fish oil as a means to supplement dietary omega (n-3) fatty acids which have demonstrated health benefits to humans with metabolic syndrome and its associated diseases. *Echium* oil has a high content of the n-3 fatty acid stearidonic acid (SDA), a precursor of the bioactive lipids eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) found in fatty cold-water fish, with known or possible functions to improve metabolism and delay the onset of or prevent diabetes. To characterize the effects of dietary Echium oil (EO) vs. fish oil (FO), the oils were formulated into either a low-fat (10% kcal; LF) or high-fat (60% kcal; HF) diabetogenic diet and fed to male C57BL/6Tac mice for 12 weeks. Compared to the low-fat or high-fat controls without the supplementation of EO or FO, EO and FO diets had no effect on blood glucose concentrations or plasma insulin levels throughout the study. The EO-enriched HF diet improved glucose tolerance by week 12 compared to the HF-CON ($p < 0.05$) and HF-FO ($p < 0.1$) groups. EO supplementation reduced visceral fat weight without affecting body mass, promoted a metabolically favorable high polyunsaturated fatty acid (PUFA) to saturated fatty acid (SFA) ratio in adipose and muscle tissues compared to the HF-CON and HF-FO diet groups, and led to higher tissue EPA and DHA

concentrations compared to both LF and HF CON ($p < 0.1$). Tissue EPA and DHA in EO were not as high as the concentrations found in mice fed the FO diets for both HF and LF. In conclusion, EO-supplemented diets in mice appear to have distinct effects from FO diets that may be exploited in future strategies to curtail metabolic disorders.

INTRODUCTION:

Type 2 Diabetes (T2D) is a major worldwide epidemic with a current prevalence of ~300 million people with an expected 50% increase by 2025 [7]. The major risk factors for diabetes are obesity and the so-called “metabolic syndrome”, whereby one-third of the U.S. population is afflicted [5]. The costs for diabetes and its complications in the U.S. alone are upwards of \$200 billion annually [5]. Lifestyle intervention plays a major role in T2D susceptibility. There is growing evidence that diets rich in specific bioactive lipids such as “omega-3” (n-3) polyunsaturated fatty acids (PUFAs) found in fatty cold-water fish have protective roles in the development of the metabolic syndrome and T2D [10,16].

The n-3 PUFAs have received considerable research focus over the last several years, especially with respect to decreased risk for cardiovascular disease, inflammatory disorders, and the metabolic syndrome, although recent meta-analyses from human clinical trials (reviewed in [18]) note some inconsistencies in regards to PUFA-associated protection from T2D among different populations. These beneficial fatty acids, enriched in fatty fish, consist principally of docosahexaenoic acid (DHA) and eicosapentaenoic acid (EPA) and serve as potent ligands activating the G-protein coupled receptor (GPCR, “GPR120”), a key receptor on cell membranes that aids in anti-inflammatory signaling, on adipocytes, and macrophages [16]. These molecules

effectively promote anti-inflammatory signaling cascades that lead to improved insulin sensitivity [11]. Insulin is exclusively made and secreted from a pancreatic endocrine cell type called β -cells, which are found in aggregates called “islets” in the pancreas. β -cell failure or loss is the underlying cause of all forms of diabetes [14]. Both diet-derived and genetically augmented tissue production of n-3 PUFAs exhibit a strong protective effect on pancreatic β -cells as demonstrated by mouse transgenic $\Delta 3$ -desaturase overexpression studies [1]. These lipids show promise to ameliorate or prevent both T1D [1] and T2D [17] due to their effectiveness in reducing inflammation and promoting insulin secretion [9]. Importantly, it appears that the proportion of the “omega-6” (n-6) PUFAs to n-3 PUFAs is key to maintaining β -cell protection from inflammatory mediators [19]. However, this may be a challenging feat with current fish oil dietary interventions, due to several aversive effects found upon fish oil supplementation such as stomach discomfort, bad taste, and the fact that it is not vegetarian-friendly [18].

Recently, there has been significant interest in identifying sustainable plant sources rich in n-3 PUFAs, or their precursors, as an alternative to fish and fish oil supplements [17]. One of the more promising plants identified is *Echium plantagineum* with stearidonic acid (SDA, C18:4 n-3) instead of ALA (18:3 n-3) as a large constituent of the n-3 PUFAs in the seed oil. SDA is central in the biosynthetic pathway leading to the potent n-3 PUFAs in fish oil, DHA and EPA. In mammals, however, it appears that only EPA is metabolized in physiologically significant amounts from SDA [17]. Clinical studies suggest that an SDA consumption of $> 9\text{g/d}$ ($\sim 130\text{ mg/kg/d}$) may be necessary to observe positive health effects due to its conversion efficiency [17]. Although there is no pre-clinical data to date on the efficacy of dietary SDA on metabolic outcomes, SDA supplementation is a promising strategy to test the effects of plant derived n-3

lipid precursors to improve metabolism, and decrease the severity, or perhaps prevent diabetes development.

The goal of this study was to determine if the dietary *Echium* oil-derived n-3 PUFA SDA is comparable to fish oil-derived EPA and DHA with respect to improving metabolic outcomes in a high saturated fat diet-induced obesity mouse model. Since *Echium* oil supplementation, as opposed to fish oil, has been reported to significantly lower certain plasma lipids associated with the metabolic syndrome in humans [2, 8, 10, 17] as an initial step in characterizing potential differences in how fish vs. plant sources of dietary n-3 PUFAs impact whole body energy physiology, we compared glucose homeostasis and tissue-specific fatty acid partitioning between *Echium* and fish oil as a function of a low- vs. high-fat diet.

MATERIALS AND METHODS:

Animals and feeding regimen. Six week-old male C57BL/6NTac mice (B6; Taconic), previously weaned onto a control chow (LabDiet 5001; 13.5% fat) and housed in the UVM Animal Facility, were *ad libitum* fed either a low-fat (Research Diets D12450B; 10% kcal from fat) or a high-fat diet (Research Diets D14292; 60% kcal from fat) supplemented with n-3 PUFAs for 12 weeks (Table 1). *Echium* oil (EO; 20% of total fat content), menhaden fish oil (FO; 20% of total fat content; positive control n-3 PUFA source), or no supplement (CON; negative control; diabetogenic diet) were formulated separately into either the high-fat or low-fat diet (Table 2). The mice were grouped randomly in cages of five individuals and subjected to one of the six diets (n=10). The guidelines specified by the UVM Institutional Animal Use and Care Committee were strictly followed for these studies.

Metabolic and glucose homeostasis assessments. Body weight, fed blood glucose concentration (FreeStyle monitor; Abbott) and plasma insulin (ELISA; AlpcO) values were measured weekly. Intraperitoneal glucose tolerance tests (IPGTT), an index of glucose clearance as a function of both glucose uptake (primarily by skeletal muscle) and pancreatic β -cell function, was carried out at 4, 8, and 12 week time-points. Following a 12 h fast, mice (n=5) were given an IP bolus of glucose (2g/kg) and blood glucose was measured at 0, 15, 30, 60, 90, and 120 minutes. For intraperitoneal insulin tolerance tests (IPITT), an index of insulin sensitivity, a different group of mice (n=5) was tested at 8 and 12 weeks. Following a 12 h fast, mice were given insulin at 0.75U/kg by an IP injection, and blood glucose was measured at 0, 15, 30, 60, 90, and 120 minutes.

Tissue collection. At 12 weeks, mice were euthanized with a lethal dose of pentobarbital (Sleepaway), and pancreas, liver, epididymal fat, quadriceps muscle, spleen, and blood were rapidly collected. The liver, pancreas, and fat pad were weighed for comparisons between groups. Tissues were snap frozen in liquid nitrogen and subsequently stored at -80°C prior to analyses, whereas heparinized blood was immediately separated into cells and plasma fractions before storing at -20°C .

Fatty acid analysis of feed. Food consumption was determined biweekly and samples from the refusals and fresh food were removed and stored at -20°C to determine the fatty acid profile and any potential degradation (i.e., oxidation) of the fatty acids of the feed. There was no difference between the refusals and the fresh food. Fatty acid content and profile of the feed samples were analyzed using a modified version of the direct transesterification method developed by Sukhija and Palmquist (1988) [15]. In brief, 1 mL of internal standard (1 mg C13:0 TAG/mL acetone), 2 mL of toluene, and 2 mL of 2% methanolic H_2SO_4 acid were added to 500 mg of ground feed

composites samples. The solution was heated at 50° C overnight. After cooling the samples to room temperature, 5 mL of 6% KHCO₃ solution and 1 mL of hexane were added. The samples were mixed and centrifuged at 500 x g for 5 min. The resulting hexane layer was dried and cleaned over a mixture of sodium sulfate and charcoal. An aliquot of the solution, containing the fatty acid methyl esters (FAME), was taken for gas chromatograph (GC) analysis. The fatty acid composition of the differing treatment groups is shown in Table 2.

Fatty acid analysis of adipose tissue. 3.75 mL of a chloroform/ methanol (1:2) mix was added to pre-weighed homogenized adipose tissue, sonicated and vortexed thoroughly to fully break down the tissue. 2.5 mL of chloroform and 1.25 mL of a 2% sodium chloride solution were added. After vortexing, and centrifuging to separate the layers, the lower chloroform layer containing the lipids was dried over anhydrous sodium sulfate. The chloroform phase was then dried under a stream of N₂ and the resulting lipid extract was transesterified using 0.5 M sodium methoxide. The resulting fatty acid methyl esters (FAME) were subjected to the GC.

Fatty acid analysis of muscle. Muscle tissue was homogenized in 5 mL methanol using an Ultra-Turrax. 5 mL of chloroform was added and the mixture was vortexed then sonicated in a water bath for 15 min before being shaken over night to obtain full denaturation of the cell membranes. After 12 hours, 5 mL of 2% sodium chloride solution and 5 mL chloroform were added. The sample was then centrifuged for 15 minutes at 8°C at 4000 rpm. The chloroform layer was dried over anhydrous sodium sulfate and evaporated under a stream of N₂ to obtain the lipid extract. Lipids were transesterified using a two-step transesterification process using 0.5 M sodium methoxide and 10% boron trifluoride in methanol. The resulting FAME solution was analyzed on a GC-2010 gas chromatograph (Shimadzu, Kyoto, Japan) equipped with a split injector, a flame ionization detector, an autosampler (model AOC-20s; Shimadzu), and a 100 m CP-Sil 88

fused-silica capillary column (100 m × 0.25 mm i.d. × 0.2 µm film thickness; Varian Inc., Palo Alto, CA) The injector and detector were both maintained at 250°C. Hydrogen was used as carrier gas at a linear velocity of 30 cm/sec. The sample injection volume was 1 µL at a split ratio between 1:100 and 1:5. The oven program used was: initial temperature of 45°C held for 4 min, programmed at 13°C/min to 175°C held for 27 min, then programmed at 4°C/min to 215°C held for 35 min. Integration and quantification was based on the FID response and achieved with GCsolution software (version 2.30.00, Shimadzu, Kyoto, Japan). Identification of FAME was accomplished by comparison of relative retention times with commercial FAME standards (37-Component FAME mix, cis/trans FAME mix, and PUFA No. 3 mix from Supelco Inc., Bellefonte, PA, USA; CLA mixture #UC-59 M from Nu-Chek Prep. Elysian, MN USA). The fatty acid results were expressed as percentages (weight/weight) of fatty acids detected with a chain length between 10 and 24 carbon atoms. The lowest level of detection was <0.001g/100g fatty acids.

Statistical analysis. Statistical analyses were performed with JMP Pro 11.0 software (SAS Institute Inc., Cary, NC). The results are expressed as mean ± SEM. A two-way ANOVA (with Tukey's post hoc test) was used to compare fat level and treatment groups for all basal and endpoint data, as well as muscle and adipose tissue fatty acid composition. Glucose homeostasis data were analyzed and displayed using GraphPad Prism. Significance was determined at $p < 0.05$ and a trend was considered at $p < 0.1$.

RESULTS

Body weights and feed intake. The average feed efficiency was significantly different between the HF and LF diets but not within the EO and FO groups (Table 3). While total weight gain and

the feed efficiency ratio between the n-3 supplemented groups did not differ significantly within the HF and LF groups, there was a significant difference in energy intake ($p < 0.001$) in the HF-EO group. This group displayed an increased energy intake that was not reflected in net weight gain, whereas the LF-FO group had a large energy intake that resulted in a trend towards heavier mice by the end of the study. The ratios of the liver to body weight were significantly different between the HF CON and HF EO groups, with EO having a larger ratio when compared to the CON and FO groups (Table 3).

Fed plasma glucose and insulin measurements. Plasma glucose and insulin concentrations were significantly different between the HF and LF groups but not within the EO and FO treatment groups (Table 3).

IPGTT and IPITTs. Figure 1 shows the IPGTT's performed on the mice at three different time points and their corresponding area under the curve (AUC) calculations. There was significant difference ($p < 0.01$) between the HF and their respective LF counterparts but not within the EO and FO treatment groups (Figure 1, panel A and B). By week 12, the endpoint of the study, there were significant differences between the HF and their respective LF counterparts as well as between the EO group and the CON group for both the LF and HF groups. For the IPITTs (Figure 2), there were no differences within the HF or LF groups at week 8, but all HF groups exhibited reduced insulin tolerance by 12 weeks. Therefore, EO supplementation improved glucose tolerance without affecting insulin tolerance by 12 weeks (Figure 1 and 2).

Tissue fatty acid composition. Despite the focus on the concentrations of SDA found in both the EO and FO diets (Table 1), there was no detectable presence of SDA within the fatty acid compositions of either adipose or muscle tissue in any of the EO or FO diets regardless of fat

level (Table 4 and 5 respectively). For adipose tissue, the HF-EO group showed a significantly lower value for palmitic acid (16:0), stearic acid (18:0), oleic acid (18:1 n-9), vaccenic acid (18:1 n-7), and linoleic acid (18:2 n-6) compared to the other diets. Compared to FO within both fat groups, the EO group also had significantly lower concentrations of total saturated fatty acids (SFAs) in the adipose and muscle tissue. Moreover, within the HF group, EO had higher concentrations of total PUFAs than FO and CON. For EPA (20:5 n-3) and DHA (22:6 n-3), two important biomarkers of improved glucose homeostasis, in both the adipose and muscle tissues, the FO group demonstrated a significantly larger amount compared to both CON and EO in the LF and HF diets, and EO showed no significant difference when compared to CON but there was a strong trend ($p < 0.1$) illustrating an increased percentage of EPA and DHA. The EO group also displayed a significantly higher percentage of n-3 PUFAs than FO and CON in HF, and CON in LF with a strong trend ($p < 0.1$) towards a higher percentage than FO in the LF diet. The muscle tissue demonstrated no difference of n-3 fatty acids between EO and FO, but EO was significantly higher than the CON in the LF treatment. Therefore, EO group's tissues contained higher amounts of EPA and DHA compared to the CON, had a greater amount of n-3 fatty acids, and contained higher PUFAs and lower SFAs.

DISCUSSION:

The goal of this study was to determine if a SDA-rich plant source such as *Echium* oil could provide similar positive effects as fish oil on the prevention of pre-diabetes by improving glucose homeostasis through the metabolism (elongation and desaturation) of SDA to its bioactive conversion products EPA and DHA. A HF diet-induced obesity model was used as a result of their expression of traits that are similar to humans, "pre-T2D" such as reduced glucose tolerance, hyperinsulinemia, and insulin resistance [4].

The increased energy intake in the HF-EO group that is not reflected in net weight gain may be explained by a higher energy metabolism in the EO mice than in the FO mice. The HF-EO mice also exhibited a lower epididymal fat weight/ body weight ratio, suggesting that although they were eating more, extra energy was either deposited into subcutaneous fat depots, which contribute little to systemic insulin resistance [3], or perhaps contributed to muscle mass.

Mild hyperglycemia was observed in the HF diet groups with no differences among the n-3 supplemented groups compared to the HF CON group. Hyperinsulinemia, the hallmark of pre-diabetes and insulin resistance [6] was on the average 2-fold increased over the 12 week study in all HF groups compared to their respective LF groups. Whereas IPITT to measure insulin resistance demonstrated no improvements in insulin tolerance in the n-3 supplemented HF groups compared to their respective controls, the 12 week IPGTT revealed a significantly improved glucose tolerance in the EO-supplemented LF and HF groups compared to their control groups ($p < 0.05$). Hence, it appears that under these dietary conditions, EO is superior to FO at maintaining normal glucose tolerance, a function of both prevailing tissue glucose uptake activity and the β -cell's secretion of insulin [12]. This finding is consistent with previous data on EO's effect on glucose disposal by Kavanagh [10] where they established that glucose disposal improved in insulin resistant monkeys on an EO diet. This improvement was not seen at the earlier time-points suggesting that it may take a few weeks for dietary supplements to affect glucose disposal, although this delay was not seen in the study of Kavanagh's et al., where the glucose tolerance was noticed by the six-week mark [10]. This disparity may reflect the difference in species, and the slightly higher percentage of GLA found in the EO diet. The 8-week IPITT revealed that the HF-FO group transiently improved their insulin sensitivity, as by 12 weeks, their values were no different from the HF-CON group.

Both the adipose and muscle tissue fatty acid analyses displayed distinct and intriguing fatty acid deposition patterns. There was no SDA detected in either of the tissues, which is at odds with some reports [2], but similar to others [10] which also was unable to detect SDA in muscle tissue. The lack of this fatty acid within the tissues may be due to the small percentage it contributed to the fat in the diets (LF-EO: 1.00% and HF-EO: 2.22% of total fatty acids), and the possibility that it was potentially converted efficiently into EPA and DHA, as seen in the increased amount found in EO compared to CON ($p < 0.1$). While the amount of DHA and EPA found in the EO diet was not as high as that found in the FO diets, this is to be expected as the FO diets contained a large amount of EPA and DHA. The EO diets, containing significantly smaller levels of EPA and DHA (not detected in LF-EO and only 0.05 g EPA/100 g of fatty acids of total fatty acids in HF-EO), depended on the metabolism of SDA to derive EPA and DHA in the tissues. These results are again similar to those found in Kavanagh's study, where an increase in n-3 PUFAs, DHA and EPA, was detected upon EO supplementation, particularly in muscle tissue [10]. This, in turn, suggests that SDA in the EO diet was likely not responsible for the improved glucose tolerance. As there was no SDA found in the adipose or muscle tissue, it is unlikely to have contributed to the enhanced glucose tolerance. However, a potential source for the enhanced glucose tolerance may be attributed to gamma-linolenic acid (GLA; 18:3 n-6). This fatty acid was in the EO diet almost to the same extent as SDA (LF-EO had 0.76 g/100 g of fatty acids and HF-EO had 1.68 g/100 g of fatty acids), but was incorporated into both adipose and muscle tissue to detectable levels that were significantly higher ($p < 0.01$ in muscle; $p < 0.001$ in adipose tissue) than the FO and CON groups. Kavanagh et al. [10] also attributed the increase in glucose tolerance found in the EO-fed group to GLA found in the tissues.

Another important difference to note was that of the percentage of SFA and PUFAs between treatment groups in the LF and HF diets in muscle tissue. According to the analysis of previous studies done by Micha and Mozaffarian [11], it has been shown that replacing SFAs with PUFAs aids in cardiovascular and diabetes prevention. The EO group demonstrated a significantly lower SFA and a higher PUFA incorporation in both muscle and adipose tissue than the CON and even the FO groups. This may be an indication of EO's potential benefits in the improvement of glucose homeostasis under conditions of a diabetogenic diet.

In summary, dietary *Echium* oil exhibits some distinct physiological effects compared to fish oil through the tissue specific incorporation of bioactive lipids and its capacity to maintain glucose homeostasis when superimposed with a diet rich in saturated fat. We have shown that dietary *Echium* oil improves glucose homeostasis, suggesting that it may (1) enhance systemic glucose uptake and possibly β -cell function, (2) leads to reduced visceral adipose accumulation, and (3) increases tissue PUFA accumulation while reducing tissue SFA accumulation. Therefore, our study indicates that *Echium* oil can improve energy metabolism at multiple levels and is a promising sustainable n-3 source with several favorable physiological attributes. However, further work is required to more fully examine its efficacy over fish oil and the underlying mechanisms.

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TABLES/ FIGURES

Table 1: Composition of experimental diets fed to mice for 12 weeks.

% of kcal	Treatment					
	LF			HF		
	CON	FO	EO	CON	FO	EO
Protein	20	20	20	20	20	20
Carbohydrate	70	70	70	20	20	20
Fat	10	10	10	60	60	60
Total	100	100	100	100	100	100
Ingredient						
Sucrose	275	275	275	275	275	275
Maltodextrin 10	500	500	500	500	500	500
Corn Starch	2025	2025	2025	0	0	0
Lard	180	144	144	2205	1764	1764
Menhaden Oil	0	36	0	0	441	0
Echium oil	0	0	36	0	0	441
Soybean Oil	225	225	225	225	225	225

Table 2: Fatty acid composition of the experimental diets fed to mice for 12 weeks (g/100 g total fatty acids, ND = not detected).

Feed Fatty Acids	Treatment					
	LF			HF		
	CON	FO	EO	CON	FO	EO
C16:0	16.15	15.87	14.95	20.29	19.75	17.76
C18:0	7.10	6.77	6.56	10.46	9.28	9.21
C18:1 n-7	26.91	24.61	25.04	34.57	29.08	30.48
C18:1 n-9	1.63	1.70	1.54	1.98	2.17	1.71
C18:2 n-6	38.80	37.75	38.62	24.16	21.50	23.26
C18:3 n-6	ND	ND	0.76	ND	0.08	1.68
C18:3 n-3	4.08	4.25	6.63	1.65	1.81	7.02
C18:4 n-3	ND	0.17	1.00	ND	0.47	2.22
C20:5 n-3	ND	1.02	ND	ND	2.39	ND
C22:5 n-3	ND	0.19	ND	0.05	0.42	0.05
C22:6 n-3	ND	0.64	ND	ND	1.84	ND
Saturated fatty acids	25.37	25.07	23.15	32.84	32.80	28.71
Mono-unsaturated fatty acids	30.57	29.36	28.40	39.74	36.87	35.09
Poly-unsaturated fatty acids	43.60	44.66	47.63	27.33	29.24	35.42
n-3	4.12	6.26	7.63	1.79	6.93	9.39
n-6	39.33	38.32	39.83	25.31	22.20	25.86
n6/n3	9.55	6.12	5.22	14.12	3.20	2.75

Table 3: Basal and endpoint measurements in LF or HF diet-fed mice (control, CON; fish oil, FO; echium oil, EO).

Variable	Treatment						SEM	Significance		
	LF			HF				Fat level (F)	Treatment (T)	F x T
	CON	FO	EO	CON	FO	EO				
Initial Body Weight (g)	19.5	19.86	19.85	19.47	19.41	19.93	1.04	NS	NS	NS
Endpoint Body Weight (g)	29.32	30.3	29.46	43.24 ^a	44.25 ^a	44.39 ^a	2.39	***	NS	NS
Weight Gain (g)	9.82	10.44	9.61	23.77 ^a	24.84 ^a	24.46 ^a	2.23	***	NS	NS
Feed Efficiency (kcal/ g wt gain/ mouse/ day)	0.019	0.018	0.018	0.029 ^a	0.03 ^a	0.029 ^a	0.002	***	NS	NS
Energy Intake (kcal/ mouse/ day)	7.90 ^a	8.96 ^b	8.18 ^c	11.71 ^d	11.68 ^d	12.25 ^e	0.13	***	***	***
Blood Glucose AUC(mg/dl) ²	1286.1	1269	1241.4	1455.7 ^{ab}	1412.8 ^a	1528 ^b	109.2	***	NS	NS
Blood Insulin AUC(ng/ml) ²	8.75	9.06	9.38	18.56 ^a	18.41 ^a	21.05 ^a	3.41	***	NS	NS
Endpoints:										
Fat Pad Weight (g)	0.77	0.63	0.60	1.49 ^a	1.48 ^a	1.38 ^a	0.27	***	NS	NS
Liver Weight (g)	1.16	1.16	1.16	1.61 ^a	1.85 ^{ab}	1.93 ^b	0.28	***	NS	NS
Fat Weight/ Body Weight (%)	2.60 ^{ab}	2.07 ^a	2.03 ^a	3.48 ^c	3.38 ^c	3.13 ^{bc}	0.75	***	NS	NS
Liver Weight/ Body Weight (%)	3.96 ^{ab}	3.87 ^{ab}	3.93 ^{ab}	3.71 ^a	4.13 ^{ab}	4.33 ^b	0.57	NS	NS	NS
*P < 0.05; **P < 0.01; ***P < 0.001										
¹ Values are means for n = 10/ group. Means in row with superscripts without a common letter differ (Tukey's <i>post hoc</i> test).										
² Fed blood glucose and insulin are reported as area under the curve (AUC) for the entirety of the 12-week study.										

Table 4: Fatty acid composition of adipose tissue (g/100g total fatty acids) in LF or HF diet-fed mice (control, CON; fish oil, FO; echium oil, EO).

Adipose Tissue Fatty Acids	Treatment						SEM	P value		
	LF			HF				Fat Level (F)	Treatment (T)	F x T
	CON	FO	EO	CON	FO	EO				
14:0	1.25 ^{AB}	1.43 ^C	1.24 ^A	0.71	1.35 ^{BC}	0.61	0.12	***	***	***
16:0	20.45	21.76 ^A	20.56	17.54 ^C	19.94	10.19 ^C	1.06	***	***	NS
16:1 n-7	9.00 ^A	8.25 ^B	8.59 ^{AB}	2.73 ^{CD}	3.34 ^C	3.30 ^D	0.71	***	NS	*
18:0	1.65	1.91 ^A	1.70	3.62 ^B	4.36 ^C	1.35 ^B	0.18	***	***	***
18:1 n-9	36.92	36.09	36.98	45.31 ^A	39.75 ^B	19.70 ^C	1.61	***	***	***
18:1 n-7	3.82 ^A	3.28 ^B	3.47 ^C	2.14 ^D	2.39 ^E	1.90 ^F	0.19	***	***	***
18:2 n-6	20.61	21.10	20.47	23.12 ^{AB}	22.56 ^A	10.93 ^B	0.93	***	NS	*
18:3 n-6	0.05	0.04	0.19 ^A	0.05	0.04	0.45 ^B	0.05	***	***	***
18:3 n-3	1.26	1.25	1.84 ^A	0.65 ^B	0.89 ^B	0.61 ^C	0.33	NS	***	***
20:5 n-3	0.03 ^A	0.08 ^B	0.04 ^{AB}	0.01 ^A	0.25	0.05 ^{AB}	0.06	**	***	***
22:5 n-3	0.04	0.10 ^A	0.06	0.03	0.20 ^B	0.09 ^A	0.03	***	***	***
22:6 n-3	0.11 ^{AB}	0.30	0.15 ^A	0.05 ^B	0.52 ^C	0.13 ^{AB}	0.09	NS	***	***
saturated fatty acids	23.82 ^A	25.62 ^B	23.95 ^A	22.30 ^C	26.27 ^B	22.00 ^C	1.22	***	***	**
mono-unsaturated fatty acids	51.94	49.66 ^A	51.12	52.10	47.41 ^B	48.88 ^A	1.47	***	***	*
poly-unsaturated fatty acids	23.09	23.68	23.66	24.96 ^A	25.45 ^A	28.35 ^B	1.17	***	***	***
-3	1.46 ^A	1.77 ^{AB}	2.14 ^B	0.78	2.00 ^B	3.25 ^C	0.46	NS	***	***
-6	21.34	21.68	21.26	23.94 ^{AB}	23.22 ^A	24.66 ^B	0.95	***	NS	*

*P < 0.05; **P < 0.01; ***P < 0.001

^A Values are means for n = 10/ group. Means in row with superscripts without a common letter differ (Tukey's *post hoc* test).

Table 5: Fatty acid composition of muscle tissue (g/100g total fatty acids) in LF or HF diet-fed mice (control, CON; fish oil, FO; echium oil, EO).

Muscle Tissue Fatty Acid	Treatment						SEM	Significance		
	LF			HF				Fat Level (F)	Treatment (T)	F x T
	CON	FO	EO	CON	FO	EO				
C14:0	1.67 ^A	1.55 ^{AB}	1.39 ^B	1.02	1.66 ^A	0.97	0.20	***	***	***
C16:0	22.47 ^{BC}	23.87 ^A	22.68 ^{ABC}	21.70 ^C	23.66 ^{AB}	21.70 ^C	1.34	NS	**	NS
C16:1 n-7	10.97 ^A	9.65 ^{AB}	8.48 ^B	3.99	3.62	3.02	2.41	***	NS	NS
C18:0	6.89	7.41	7.40	7.42	7.52	7.85	1.78	NS	NS	NS
C18:1 n-9	19.79 ^{BC}	17.61 ^C	19.13 ^{BC}	26.35 ^A	23.14 ^{AB}	24.44 ^{AB}	5.53	**	NS	NS
C18:1 n-7	0.03	0.04 ^A	0.02 ^A	0.03 ^B	0.04 ^{BC}	0.02 ^C	0.30	***	**	NS
C18:2 n-6	12.88 ^{AB}	11.14 ^A	13.07 ^B	17.25 ^{CD}	15.81 ^C	17.86 ^D	1.97	***	*	NS
C18:3 n-6	0.05	0.05	0.06	0.04	0.04	0.27 ^A	0.10	*	**	**
C18:3 n-3	0.55 ^A	0.50 ^A	0.74 ^{AB}	0.66 ^{AB}	0.85 ^B	2.75 ^C	0.30	***	***	***
C20:5 n-3	0.12 ^A	0.36 ^B	0.15 ^A	0.11 ^A	0.71 ^C	0.24 ^{AB}	0.20	*	***	*
C22:5 n-3	0.90 ^A	1.72 ^{BC}	1.50 ^{CD}	1.20 ^{AD}	1.95 ^{BE}	2.24 ^D	0.47	**	***	NS
C22:6 n-3	8.06 ^{AC}	13.22 ^B	10.94 ^{AB}	7.17 ^C	11.77 ^B	7.68 ^{AC}	3.65	NS	**	NS
Saturated Fatty Acids	31.44	33.29	31.92	30.64 ^A	33.51 ^B	31.01 ^A	2.47	NS	*	NS
Mono-unsaturated fatty acids	36.64	32.70	33.04	34.66	31.10	31.42	6.47	NS	NS	NS
Poly-unsaturated fatty acids	30.89 ^A	32.89	34.04	33.96	34.40	36.49 ^B	4.48	NS	NS	NS
n-3	9.62 ^A	15.81 ^B	13.32 ^B	9.13 ^A	15.28 ^B	12.91 ^{AB}	3.99	NS	***	NS
n-6	21.22 ^A	16.98 ^B	20.68 ^{AC}	24.80 ^D	19.07 ^C	23.54 ^D	1.81	***	***	NS

*P < 0.05; **P < 0.01; ***P < 0.001

¹ Values are means for n = 10/ group. Means in row with superscripts without a common letter differ (Tukey's *post hoc* test).

FIGURE LEGEND:

Figure 1: Glucose tolerance tests (IPGTT) and their corresponding area under the curve (AUC). Values represent means \pm SEM. Panel A) IPGTT at 4 weeks (n=60), panel B) IPGTT at 8 weeks (n=30), and panel C) IPGTT at 12 weeks (n=30). Values represent means \pm SEM. * $p < 0.05$.

Figure 2: Insulin tolerance tests (IPITT) and the corresponding area under the curve (AUC). Values represent means \pm SEM. Panel A) IPITT at 8 weeks (n=30) and panel B) IPITT at 12 weeks (n=30). Values represent means \pm SEM. * $p < 0.05$.

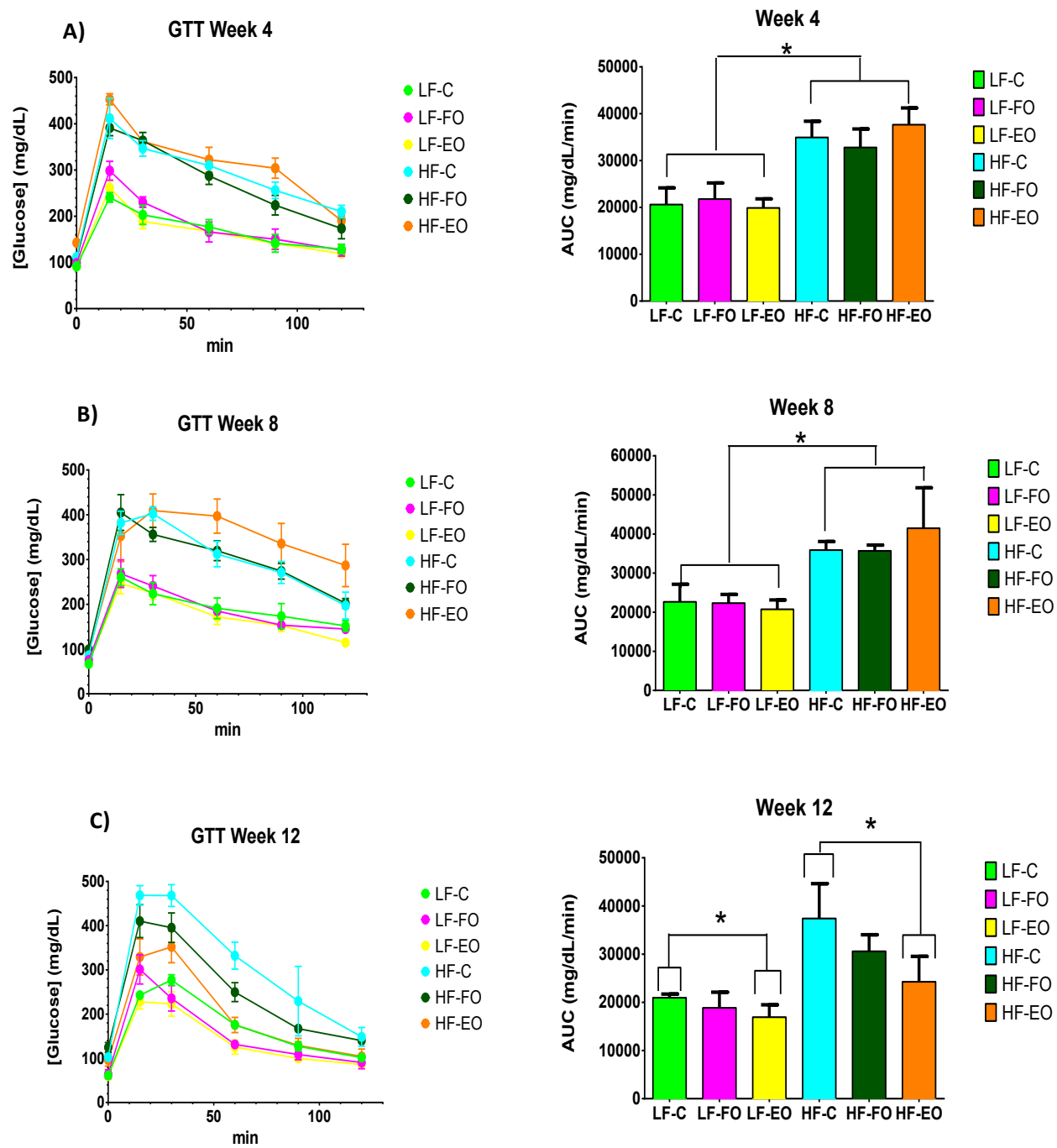


Figure 1

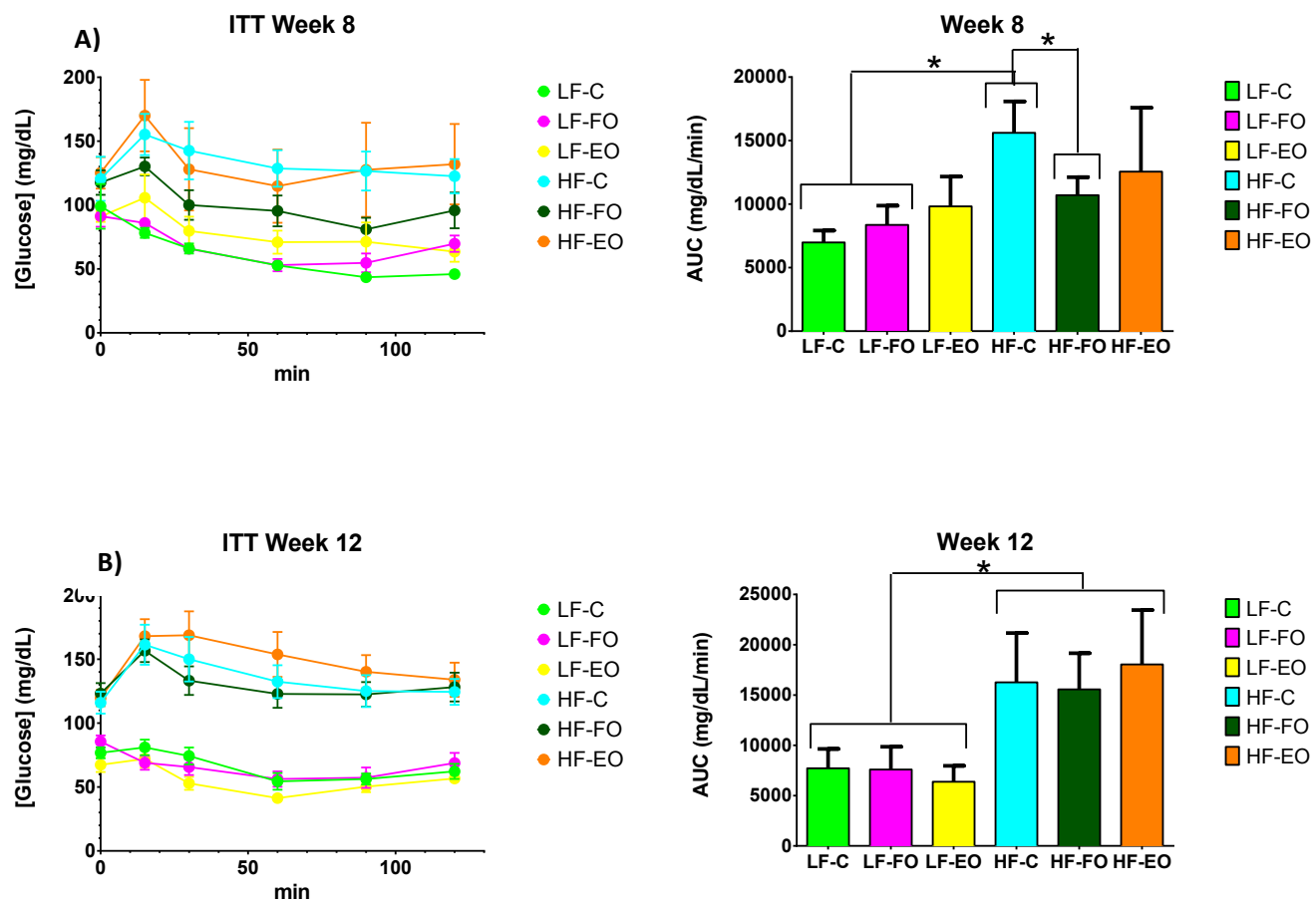


Figure 2