Supplementary Figures and Tables

Omega-9 Modifies Viscoelasticity and Augments Bone Strength and Architecture in a High-Fat Diet-Fed Murine Model

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Table S1. Starting body weight (g) and the *p* values obtained when each of the groups were compared. *denotes a significant difference.

	Control	$HFD^{50:50}$	HSF	PUFA
Control				
$HFD^{50:50}$	0.056			
HSF	0.016*	0.834		
PUFA	0.009*	0.047*	0.047*	

Table S2: Final body weight (g) and the *p* values obtained when each of the groups were compared. *denotes a significant difference

	Control	$HFD^{50:50}$	HSF	PUFA
Control				
$HFD^{50:50}$	0.009*			
HSF	0.009*	0.209		
PUFA	0.009*	0.465	0.016*	

Table S3: Total weight gain (g) over the 8-week study period and the *p* values obtained when each of the groups were compared. *denotes a significant difference

	Control	$HFD^{50:50}$	HSF	PUFA
Control				
$HFD^{50:50}$	0.008*			
HSF	0.009*	0.075		
PUFA	0.008*	0.916	0.0028*	

Table S4: Week 1 starting food intake (g) and the *p* values obtained when each of the groups were compared. *denotes a significant difference

	Control	$HFD^{50:50}$	HSF	PUFA
Control				
$HFD^{50:50}$	0.016*			
HSF	0.917	0.016*		
PUFA	0.009*	0.009*	0.009*	

Table S5: Week 8 final food intake (g) and the *p* values obtained when each of the groups were compared. *denotes a significant difference

	Control	$HFD^{50:50}$	HSF	PUFA
Control				
$HFD^{50:50}$	0.021*			
HSF	0.248	0.076		
PUFA	0.009*	0.028*	0.016*	

Table S6: Cumulative food intake (kcal) over the 8-week study period and the p values obtained when each of the groups were compared. *denotes a significant difference

	Control	$HFD^{50:50}$	HSF	PUFA
Control				
$HFD^{50:50}$	0.009*			
HSF	0.009*	0.175		
PUFA	0.009*	0.009*	0.009*	

Table S7: The biomechanical strength properties at the tibial midpoint in each of the groups. Values are unadjusted according to body weight and tibial length

Variables	*Control*	*$HFD^{50:50}$*	*HSF*	*PUFA*
Biomechanical strength properties at tibial midpoint				
Yield stress σy (MPa)	77.32 ± 10.96	85.74 ± 10.94	52.08 ± 5.27	85.28 ± 13.69
Ultimate stress σu $(MPa$	104.68 ± 19.85	122.88 ± 6.51	67.57 ± 4.55	108.69 ± 15.34
Fracture stress σf (MPa)	91.68 ± 20.74	101.98 ± 11.51	62.42 ± 5.98	87.74 ± 15.43
Elastic modulus E (GPa)	2.54 ± 0.53	1.94 ± 0.18	1.03 ± 0.19	1.80 ± 0.38

Table S8: [Unadjusted data]. A comparison of yield stress in each of the diet groups. *denotes a significant difference

	Control	$HFD^{50:50}$	HSF	PUFA
Control				
$HFD^{50:50}$	0.602			
HSF	0.251	0.028*		
PUFA	0.806	1.000	0.142	

Table S9: [Unadjusted data]. A comparison of ultimate stress in each of the diet groups. *denotes a significant difference

	Control	$HFD^{50:50}$	HSF	PUFA
Control				
$HFD^{50:50}$	0.754			
HSF	0.175	0.009*		
PUFA	1.000	0.806	0.086	

Table S10: [Unadjusted data]. A comparison of fracture stress in each of the diet groups. *denotes a significant difference

	Control	$HFD^{50:50}$	HSF	PUFA
Control				
$HFD^{50:50}$	0.917			
HSF	0.602	0.016*		
PUFA	0.806	0.327	0.221	

Table S11: [Unadjusted data]. A comparison of elastic modulus in each of the diet groups. *denotes a significant difference

	Control	$HFD^{50:50}$	HSF	PUFA
Control				
$HFD^{50:50}$	0.251			
HSF	0.028*	0.009*		
PUFA	0.327	0.806	0.086	

Table S12: Mean ± standard error BMD (g/cm^3) of whole bone and by block.

Variable	*Control*	*$HFD^{50:50}$*	*HSF*	*PUFA*
Mean BMD (g/cm^3) Whole bone	0.53 ± 0.03	0.50 ± 0.02	0.48 ± 0.02	0.51 ± 0.03
Mean BMD by block				
1	0.32 ± 0.02	0.31 ± 0.02	0.30 ± 0.02	0.26 ± 0.01
2	0.43 ± 0.02	0.41 ± 0.02	0.39 ± 0.02	0.40 ± 0.02
3	0.47 ± 0.03	0.45 ± 0.03	0.43 ± 0.02	0.46 ± 0.02
4	0.51 ± 0.04	0.49 ± 0.04	0.49 ± 0.02	0.51 ± 0.03
5	0.56 ± 0.03	0.54 ± 0.03	0.52 ± 0.02	0.56 ± 0.03
6	0.65 ± 0.02	0.61 ± 0.02	0.58 ± 0.01	0.64 ± .008
7	0.73 ± 0.03	0.70 ± .007	0.67 ± .006	0.73 ± 0.06

Table S13: Mean ± standard error BMC (g) of whole bone and by block.

Variable	*Control*	*$HFD^{50:50}$*	*HSF*	*PUFA*
Mean BMC (g) Whole bone	0.38 ± 0.02	0.31 ± 0.01	0.32 ± 0.01	0.29 ± 0.01
Mean BMC by block				
1	0.45 ± 0.05	0.37 ± 0.03	0.38 ± 0.04	0.29 ± 0.02
2	0.40 ± 0.04	0.31 ± 0.03	0.33 ± 0.03	0.29 ± 0.02
3	0.37 ± 0.05	0.31 ± 0.04	0.31 ± 0.05	0.29 ± 0.05
4	0.35 ± 0.06	0.30 ± 0.05	0.31 ± 0.05	0.29 ± 0.05
5	0.35 ± 0.04	0.30 ± 0.04	0.30 ± 0.04	0.28 ± 0.03
6	0.36 ± 0.02	0.30 ± 0.02	0.29 ± 0.02	0.28 ± 0.02
7	0.35 ± 0.01	0.30 ± 0.008	0.30 ± 0.01	0.30 ± 0.01

Table S14: Mean ± standard error BV/TV of whole bone and by block.

Variable	*Control*	*$HFD^{50:50}$*	*HSF*	*PUFA*
Mean BV/TV Whole bone	0.59 ± 0.02	0.62 ± 0.02	0.56 ± 0.02	0.59 ± 0.02
Mean BV/TV by block				
1	0.43 ± 0.03	0.50 ± 0.03	0.42 ± 0.04	0.39 ± 0.03
2	0.53 ± 0.07	0.58 ± 0.07	0.50 ± 0.06	0.52 ± 0.06
3	0.57 ± 0.06	0.61 ± 0.04	0.53 ± 0.04	0.56 ± 0.04
4	0.61 ± 0.06	0.62 ± 0.05	0.59 ± 0.03	0.62 ± 0.05
5	0.62 ± 0.04	0.65 ± 0.04	0.61 ± 0.04	0.63 ± 0.05
6	0.66 ± 0.01	0.66 ± 0.02	0.61 ± 0.02	0.65 ± 0.02
7	0.72 ± 0.03	0.72 ± 0.01	0.67 ± 0.01	0.74 ± 0.05

Table S15: Tibial bone mineral and volume levels in the antero-posterior and medio-lateral aspects. Data obtained from 1 animal per group.

Variables	*Control*	*HFD$^{50:50}$*	*HSF*	*PUFA*
AP-ML tibia bone mineral and volume levels (sum)				
BMD (mg.cm^{-2}):				
Anterior	3.79	3.70	3.57	3.55
Lateral	3.98	3.82	3.52	3.81
Medio	3.38	3.23	3.19	3.33
Posterior	3.55	3.31	3.26	3.54
BMC (mg):				
Anterior	3.09	2.59	2.63	2.31
Lateral	2.7	2.22	2.24	2.04
Medio	1.87	1.59	1.63	1.54
Posterior	2.88	2.33	2.38	2.21
BV/TV%:				
Anterior	4.55	4.81	4.39	4.41
Lateral	4.35	4.46	3.98	4.27
Medio	3.85	4.11	3.75	3.93
Posterior	3.80	3.97	3.63	3.84

Table S16: BMC in each of the four planes in mice in the HFD$^{50:50}$ group. *denotes a significant difference

	Lateral	Anterior	Medial	Posterior
Lateral				
Anterior	0.048*			
Medial	0.003*	0.002*		
Posterior	0.338	0.142	0.003*	

Table S17: BMC in each of the four planes in mice in the PUFA group. *denotes a significant difference

	Lateral	Anterior	Medial	Posterior
Lateral				
Anterior	0.035*			
Medial	0.018*	0.006*		
Posterior	0.035*	0.338	0.009*	

Table S18: BMC in each of the four planes in mice in the HSF group. *denotes a significant difference

	Lateral	Anterior	Medial	Posterior
Lateral				
Anterior	0.085			
Medial	0.004*	0.003*		
Posterior	0.482	0.142	0.004*	

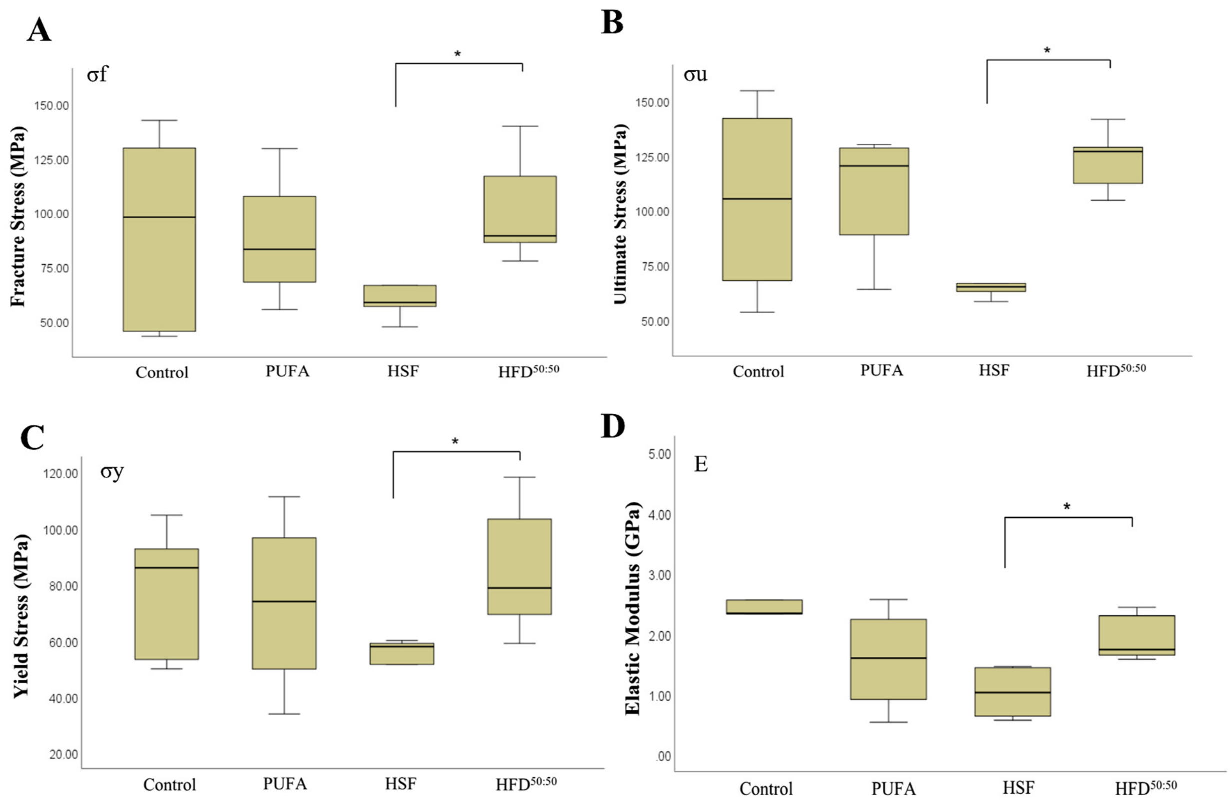

Figure S1. [A-D] The mechanical properties of yield stress σ_y, ultimate stress σ_u, strength σ_f and elastic modulus E at the tibial mid-point, varied between the different diet groups. The data has not been adjusted to body weight

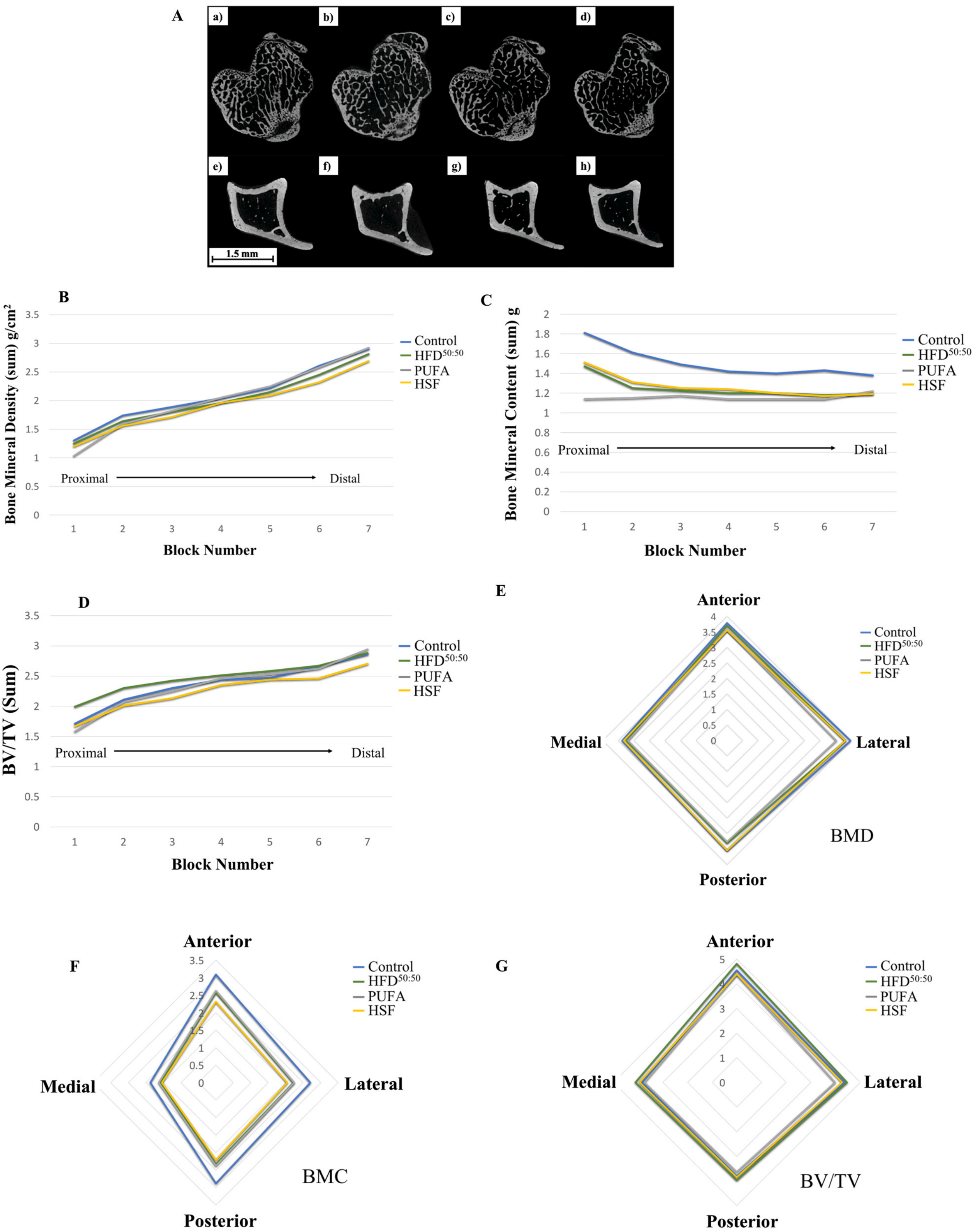

Figure S2. [A(a, b, c, d)] Representative transverse μCT images showing the tibial cancellous bone region in the same location in the control, HSF, HFD$^{50:50}$, and PUFA diet groups respectively. Trabecular bone loss is noted in all high-fat diet groups when compared with the control fed animal. [A(e, f, g, h)] representative transverse μCT images showing the tibial mid-cortical bone region in the same location in the control, HSF, HFD$^{50:50}$, and PUFA diet groups respectively. [B] BMD, [C] BMC and [D] BV/TV% from the proximal to distal region of the tibia. [E] BMD, [F] BMC and [G]

BV/TV% in the AP/ML sectors. The BV/TV fraction measured in the HFD$^{50:50}$ group, supports the histomorphometry result obtained (n=1).

No significant differences in BMD were found. However, BMC was significantly higher in the chow fed animal when compared with the HFD$^{50:50}$, HSF and PUFA groups (p = 0.002, 0.007 and <0.0001 respectively). The BV/TV% fraction was highest in the HFD$^{50:50}$ group and significantly increased when compared with the HSF group only (p = 0.017). When levels of BMD were evaluated from the proximal (block 1) to distal (block 7) regions along the tibia [**Supplementary Figure S2** B and **Supplementary Table S12**], results showed lowest values in the HSF group, with a significantly less BMD found in block 6 when compared with the control (p = 0.043) and PUFA (p = 0.021) groups. In block 7, significantly lower BMD was measured in the HSF-fed animal when compared with those who received a HFD$^{50:50}$ diet (p = 0.021). No other significant differences were found. Bone mineral content was demonstrated to increase from block 1 – 7 in the control fed animal when compared to each of the high-fat diet groups. A significantly lower BMC was measured in the PUFA group when compared with the control-fed mouse in block 1 (p = 0.043) [**Supplementary Figure S2** C and **Supplementary Table S13**]. In block 7, BMC was significantly lower in all high-fat diet groups when compared with the control fed animal (HFD$^{50:50}$, p = 0.021; HSF, p = 0.043; PUFA, p = 0.043). No other significant differences were found. The BV/TV fraction varied along the length of the tibia and results showed an increased BV/TV% in all 7 regions in the HFD$^{50:50}$ group when compared to all other groups [**Supplementary Figure S2** D and **Supplementary Table S14**]. Significantly increased values were demonstrated in block 7 and when compared with the HSF group (p = 0.021). No other significant differences were found. When BMD, BMC and BV/TV% were compared in the antero-posterior and medio-lateral planes, results showed increased BMD in the control fed animals in all four aspects when compared with all animals in the high-fat diet groups, however no significant differences were found. In all groups, BMD was highest in the lateral aspect and lowest medially [**Supplementary Figure S2** E and **Supplementary Table S15**]. In control fed mice, BMC levels were significantly increased anteriorly when compared with the lateral, medial and posterior regions of the tibiae (p = 0.002 in all cases; **Supplementary Figure S2** F). A similar trend was observed within animals in each of the high-fat diet groups (statistical comparisons are presented in **Supplementary Tables S16-S18**). Significantly increased BMC was measured in the control fed mice when compared in the lateral (HFD$^{50:50}$, p = 0.013; HSF, p = 0.018; PUFA p = 0.002) and anterior (HFD$^{50:50}$, p = 0.025; HSF, p = 0.035; PUFA p = 0.035) aspects when compared with the high-fat fed animals. Posteriorly, significantly increased BMC was measured in the control group when compared with PUFA fed animals (p = 0.002). No other significant differences were found. Animals in the control group demonstrated a significantly increased BV/TV fraction in the anterior sector when compared with the medial (p = 0.025) and posterior (p = 0.048) sectors [**Supplementary Figure S2** G]. This trend was observed in each of the high-fat diet groups however, no significant differences were found.