MS ID#: JLR/2010/013144 Revision #2

Supplemental Figures

The Intestine Maybe a Major Site of Action for the ApoA-I Mimetic Peptide 4F Whether the Peptide is Administered Subcutaneously or Orally

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Abbreviated Title: *4F may act in the intestine regardless of route of administration*

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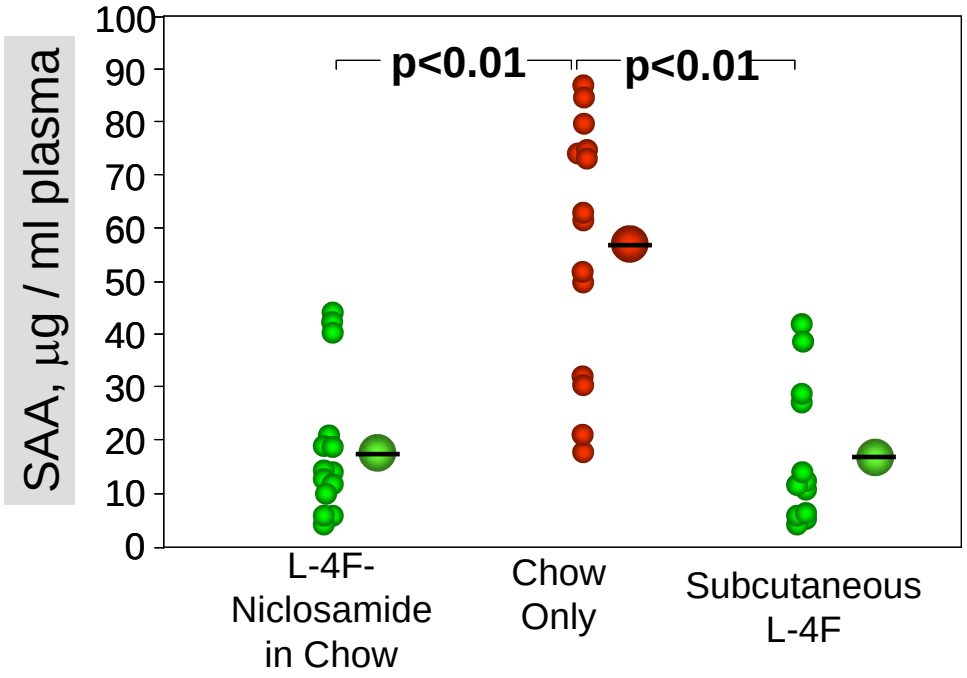
Supplementary key words: atherosclerosis ● apolipoprotein A-I ● apolipoprotein A-I mimetic peptides ● lipoproteins ● HDL

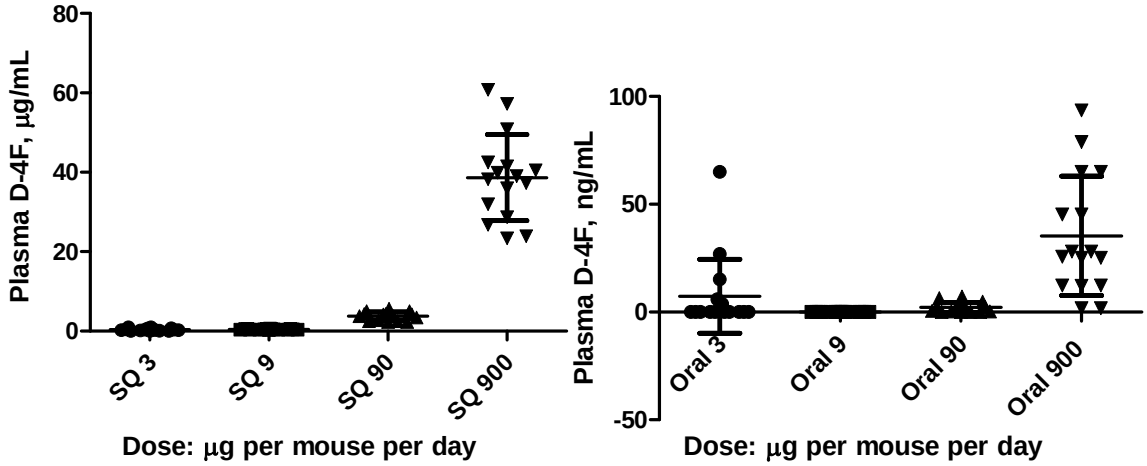
Abbreviations: CHD, coronary heart disease; Cmax, maximal concentration; HII, HDL-inflammatory index; IV, intravenous; SAA, Serum amyloid A; SQ, subcutaneous; 4F (Ac-D-W-F-K-A-F-Y-D-K-V-A-E-K-F-K-E-A-F-NH_2); D-4F, 4F synthesized from all D-amino acids; L-4F, 4F synthesized from all L-amino acids; Sc-D-4F, (Ac-D-W-F-A-K-D-Y-F-K-K-A-F-V-E-E-F-A-K-NH_2) a control scrambled peptide containing the same D-amino acids as in D-4F but in a sequence that does not promote α-helical formation; LPA, lysophosphatidic acid.

Supplemental Figure 1.



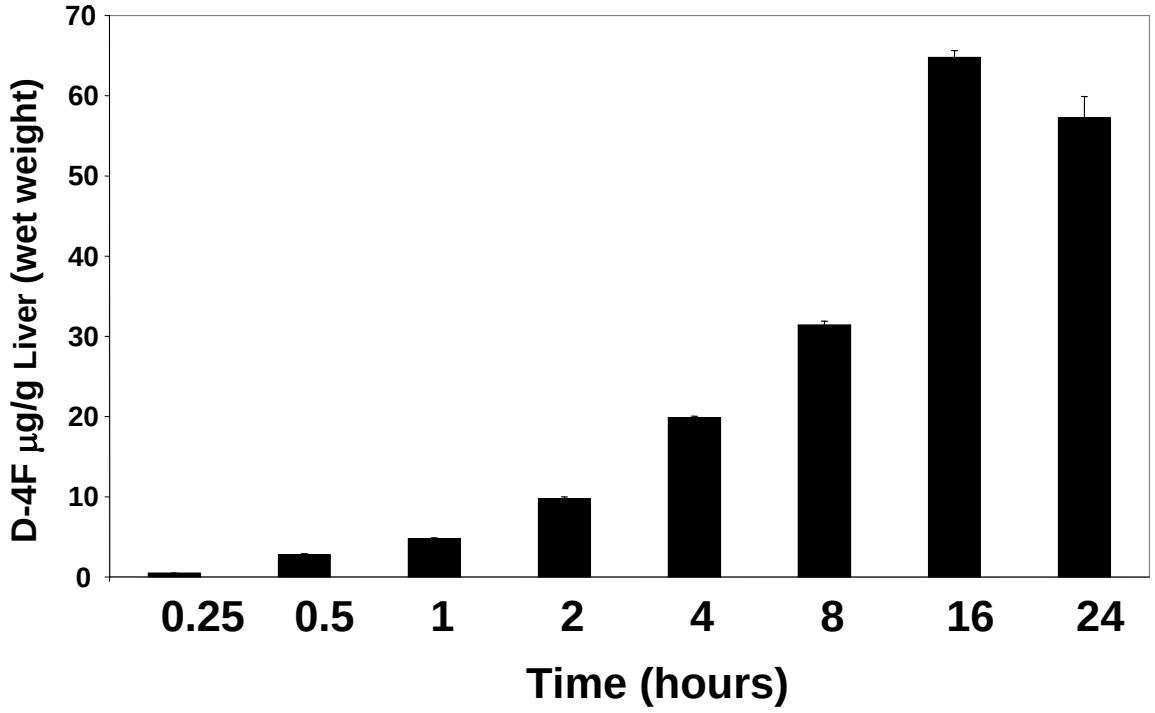
Supplemental Figure 1. SAA levels were reduced similarly in mice administered L-4F with niclosamide orally compared to the same dose of L-4F administered by subcutaneous (SQ) injection without niclosamide. Female apoE null mice (n = 12 -14 per group) age 16 – 18 months were administered mouse chow with or without L-4F (200 µg/mouse/day; 10 mg/kg/day) with niclosamide 2,000 µg/mouse/day or were administered L-4F by SQ injection at a dose of 10 mg/kg/day without niclosamide. After one week the mice were bled and serum amyloid A (SAA) levels were determined by ELISA as described in Methods.

Supplemental Figure 2.



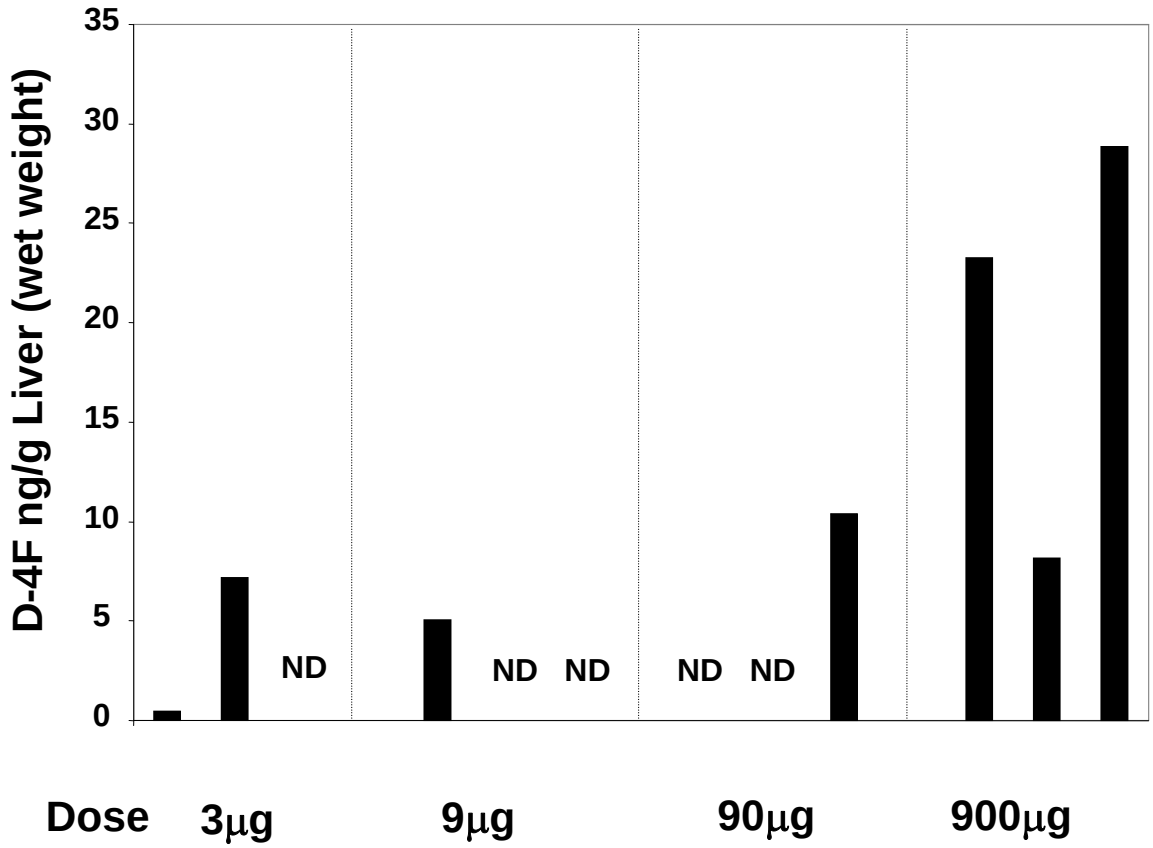
Supplemental Figure 2. The plasma D-4F levels of the mice described in Figure 4 and Table 2 are shown by the symbols which represent individual values for each mouse that received D-4F; the longer horizontal line represents the Mean and the shorter horizontal lines define one SD above and below the Mean.

Supplemental Figure 3.



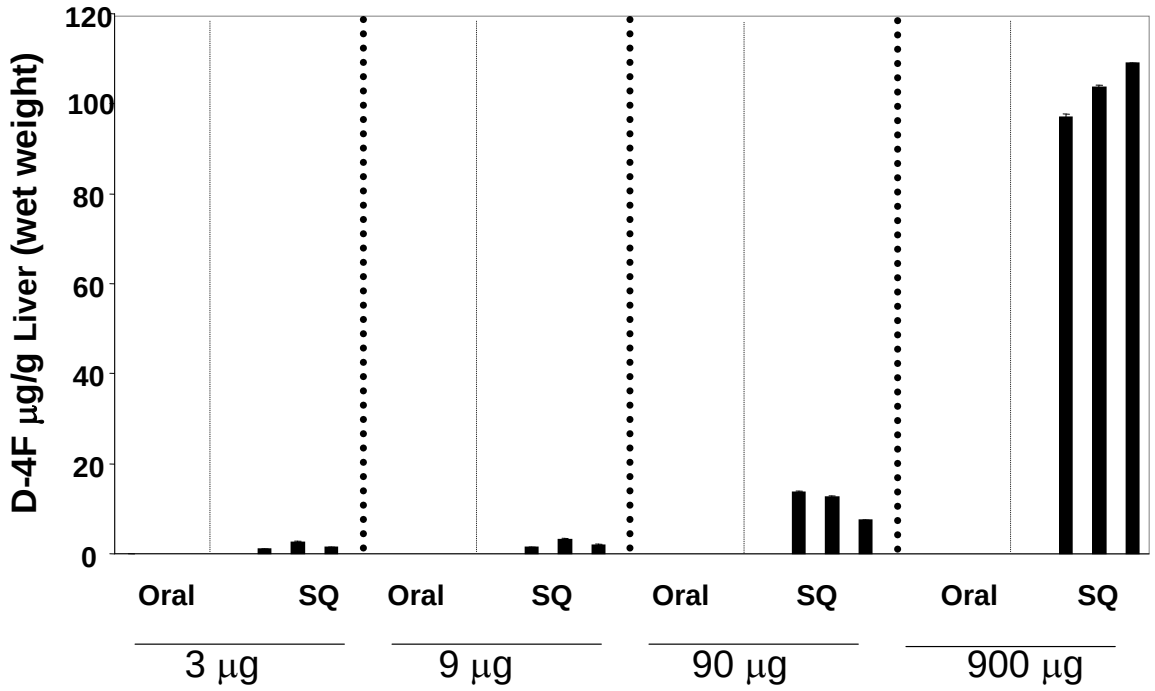
Supplemental Figure 3. Time course for hepatic D-4F levels after SQ administration. Eight female apoE null mice 6 – 7 months of age were fed a Western diet for two weeks and were administered 900 μg of D-4F SQ. The liver from one mouse at each time point was purged of blood and harvested and hepatic D-4F levels were determined as described in Methods. The values shown are the Mean ± SD of three samples from the liver of each mouse.

Supplemental Figure 4A.



Supplemental Figure 4A. Dose response for hepatic D-4F levels after oral administration. Twelve female apoE null mice 6 – 7 months of age were fed a Western diet for two weeks. The mice were administered the dose of D-4F in their drinking water shown on the X-axis. Sixteen hours after the peptide was consumed, the livers from 3 mice at each time point were purged of blood, harvested and hepatic D-4F levels were determined as described in Methods. The values shown are the individual values for each of the 3 mice at each dose. ND = not detected (i.e. the values were below the level of quantification of the assay).

Supplemental Figure 4B.



Supplemental Figure 4B. Dose response for hepatic D-4F levels after SQ or oral administration. Female apoE null mice 6 – 7 months of age were fed a Western diet for 2 weeks and were administered the dose of D-4F SQ as shown on the X-axis. Sixteen hours after the administration of the peptide, the livers from 3 mice at each SQ dose were purged of blood, harvested, and hepatic D-4F levels were determined as described in Methods. The values shown are Mean ± SD for each mouse at each SQ dose determined in triplicate samples. The mean values for the 3 mice in each dose group in Figure 4A were also calculated and plotted (Oral). Since the hepatic D-4F levels in all of the mice in Figure 4A were less than 1 μg/g liver, these values are not visible in the graph.