THE EFFECTS OF DIETARY FAT ON HEMOSTASIS

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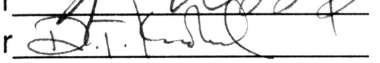
Honors Director 

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INTRODUCTION

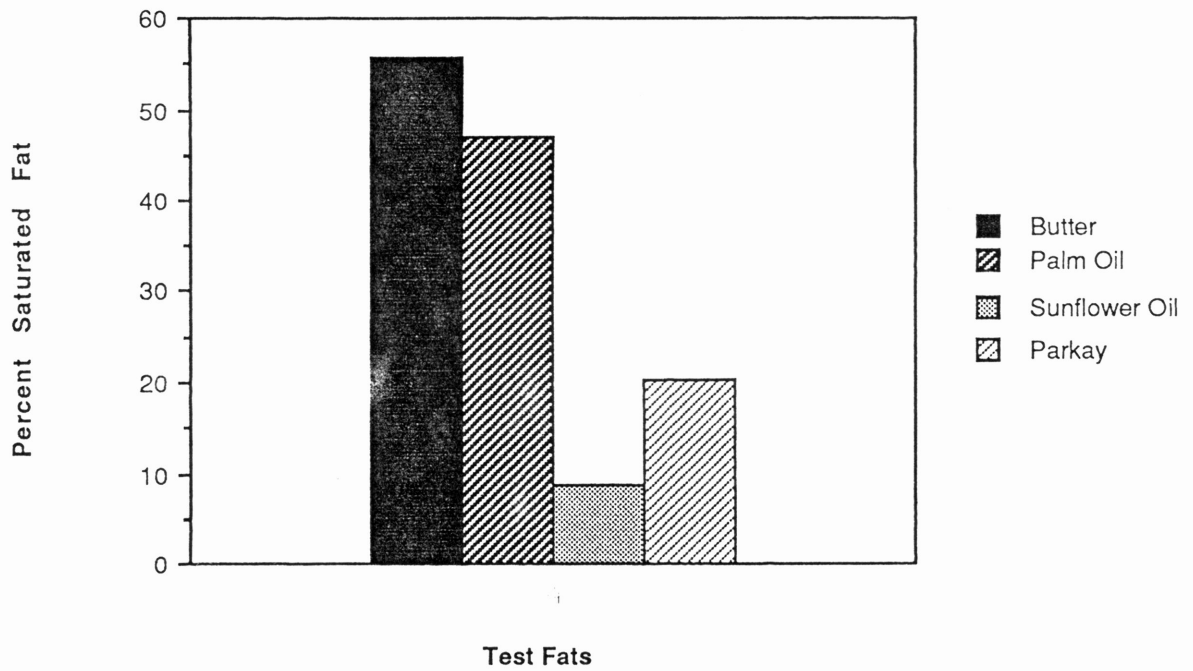
In recent years, concern over diet has grown tremendously due to the discovery of a possible correlation between diet and heart disease. Diets high in saturated fat are thought to increase a person's likelihood to have a heart attack or a stroke. This is because some saturated fats appear to increase serum cholesterol levels, and high cholesterol levels often lead to heart disease. Diets that are high in saturated fats may elevate serum cholesterol levels. When patients with high cholesterol levels change their diets to avoid saturated fats, usually decreasing total dietary fat as well, cholesterol levels often come down (1).

Fats and oils contain a variety of fatty acids. Some of these fatty acids are saturated; all their carbon atoms are completely saturated with hydrogen. Other fatty acids are unsaturated. These acids contain at least one carbon/carbon double bond. Unsaturated fats, such as sunflower oil, safflower oil, soybean oil, and some margarines contain only 5-20% saturated fatty acids. Palm oil, which is 47% saturated, is considered to be a saturated fat. It is popularly considered to be as bad for you as butter, which is 55.7% saturated (2), and coconut oil, which is 92%

saturated (3)(Figure 1). Hornstra's lab, however, in experiments with rats and rabbits showed that dietary palm oil behaved more like sunflower oil than coconut oil in its effects on hemostasis. Both the palm oil diet and sunflower oil diet showed distinct antithrombotic effects (4-6).

Prostaglandin I_2, or prostacyclin, and thromboxane A_2, both derivatives of arachidonic acid, have important blood clotting effects. Prostacyclin is a potent inhibitor of platelet aggregation, preventing clots from forming, while thromboxane A_2 is a strong inducer of platelet aggregation (7,8). Often the ratio of thromboxane A_2/prostacyclin is used as an indication of the blood's tendency to clot (4). O'Dea found that rats placed on a butter diet for three weeks had decreased prostacyclin production, indicating a higher thromboxane A_2/prostacyclin ratio and an increased tendency for blood clotting (9). Hornstra's lab, however, showed that rabbits eating a diet of 32% palm oil for 1.5 years had a decreased ratio when compared to rabbits eating a 32% sunflower oil diet. This result indicated that the palm oil diet reduced the blood clotting tendencies in the rabbits (4). He also demonstrated a similar blood clotting time in rats on a palm oil diet and rats on a sunflower oil diet. These clotting times were much longer than those of the rats on a coconut oil diet (5,6).

Figure 1: Percent saturated fat in each test fat.



This initial experimentation with animal models indicated that dietary palm oil did not behave as a saturated fat but rather as an unsaturated fat. Because little research with palm oil has been performed with human subjects, the following experiment involving a human dietary study to compare the effects of palm oil to sunflower oil, butter and margarine was performed. Blood samples taken from the subjects were used to determine the levels of prostacyclin and thromboxane A_2. The normal levels of prostacyclin and thromboxane A_2 in the blood are less than 5 pg/ml (8,10,11). It is therefore necessary to use a sensitive assay such as radioimmunoassay to measure these compounds. In a radioimmunoassay, a radiolabeled compound competes with an identical naturally occurring compound in the sample for binding sites on an antibody. Unbound components are seperated from the bound components, and the bound componenets are measured for radioactive activity by a gamma scintillation counter. The more the sample compound binds, the less the radioactive compound is able to bind. A standard curve is prepared using standards of known concentrations. When these standards are plotted versus the radioactive activity, a curve is produced which can be used to determine the concentration of a sample. This technique is senstitive, it is able to detect levels of prostacyclin and thromboxane A_2 as low as 5

pg/ml, and has little problem with cross-reactivity (12). It was therefore used to determine prostacyclin levels and thromboxane A_2 levels in the serum of the subjects in this study. The hypothesis of the experiment was that palm oil could behave as sunflower oil with both diets having antithrombotic effects.

MATERIALS AND METHODS

Subjects and Diet

Thirty male subject between the ages of thirty and sixty with normal cholesterol levels and normal blood pressure levels were placed on six diets. Each of the six diets used a specific test fat to derive approximately 60% of the fat intake or 24% of the total caloric intake. The test fats were incorporated into a fat spread, milk, ice cream, and cookies. The test fats used were

1) butter
2) crude palm oil
3) refined, bleached, and deodorized (RBD) palm oil
4) RBD sunflower oil
5) Parkay margarine
6) 80% RBD palm oil + 20% RBD sunflower oil

The diet period lasted for six weeks, and each subject had to keep a diet record for part of the diet period to insure he was eating the correct percentage of test fat.

Blood Collection and Storage

Blood samples were collected from each subject the week prior to the diet period in order to establish a baseline and during weeks one, three, five, and six of the diet period. The blood was collected in 7 ml vacutainer tubes containing EDTA, an anticoagulent. Immediately after collection, 0.07 ml of a 0.4% aspirin solution was added to each tube to act as a prostaglandin synthase inhibitor (13). The tubes were then centrifuged at 6000xg for fifteen minutes. The plasma was pipeted off and stored at -70°C in plastic tubes until the time of the assay.

Radioimmunoassays

The two compounds of interest, prostaglandin I_2 and thromboxane A_2, have short half-lives and must be measured indirectly through their stable metabolites 6-keto prostaglandin $F_{1\alpha}$ and thromboxane B_2, respectively. Radioimmunoassay kits for these two metabolites were obtained from Biotecx Laboratories, Inc., and their procedures for the radioimmunoassays of the samples and standards were followed. Six samples were run in triplicate to insure repeatability.

RESULTS

All samples for both the 6-keto prostaglandin $F_{1\alpha}$ assay and the thromboxane B_2 assay had radioactive counts higher than the counts for

the zero standard. This is probably the result of non-specific binding occurring in the samples. Something in the plasma, in addition the the rabbit antiserum of the radioimmunoassay kit, could have bound the radioactive and sample 6-keto prostaglandin $F_{1\alpha}$ and thromboxane B_2 (Tables I and II).

Normally, a standard curve, such as the curve in Figure 2, plotting the radioactive counts per minute of various standards versus their respective metabolite concentrations is used to determine the concentration of the metabolite in the sample. Because none of these samples had counts per minute in the range of a standard curve, the average counts per minute for each diet were plotted versus weeks of the diet study in order to show any change in binding.

The samples in weeks one and three of the thromboxane B_2 assay were incubated for a shorter time than those of weeks zero, five, and six. The samples incubated for shorter times had lower radioactive and sample binding. Similarly incuabated standards, however, still produced a zero standard with less radioactive binding than the samples. The thromboxane B_2 radioactive counts per minute varied from week to week, but overall the change in counts per minute was minimal (Table I). The butter, crude palm oil, sunflower oil, and sunflower/palm oil mix diets showed a

Table I: 6-keto prostaglandin $F_{1\alpha}$ radioimmunoassay counts per minute by diet

		WEEKS OF DIET				
		0	1	3	5	6
	1	12476	-------	12798.9	13084.7	13020
	2	12636.2	11782	12937.7	13021.7	12760.4
BUTTER	3	12415	11501.3	-------	11350.3	12165
DIET	4	13122.9	-------	12267.8	13988	13358.2
	5	13945.9	13288	12687.8	-------	13118.9
Average		12919.2	12190.4	12673.1	12861.2	12884.5
CRUDE	1	11124.7	13412.7	12337.8	12395.9	12173.8
PALM	2	12523.1	13364.2	12719.7	12428.7	12260.7
OIL	3	12581.2	13408.6	13197	11664.6	13062
DIET	4	12895	12878.1	13284.1	12873.5	11918.8
Average		12281	13266.2	12884.7	12340.7	12353.8
REFINED	1	11260.5	11119.6	12332	12279	11251.3
PALM	2	10668.5	13012.7	-------	11802	12185.1
OIL	3	13537.1	14030	13478	12661.3	12445.2
DIET	4	13167	12686.3	12442.1	12376.9	12304.8
Average		12158.3	12712.2	12751.0	12280.1	12046.6
SUN-	1	13763.3	13075.8	14023	13615.3	12415.4
FLOWER	2	13118.6	12743	11534	13439.4	12477
OIL	3	13512	12977	12949	13397.3	12296
DIET	4	-------	12764.8	11895.3	13128	-------
	5	13086.8	13924.7	14182	13395	11589.1
Average		13370.2	13097.1	12916.7	13395	12194.4
	1	12847.5	11533	10866.5	10577.7	10634.6
	2	13730	11486	12029.8	12600.2	12626
PARKAY	3	14135.5	14509.7	11887.1	-------	-------
DIET	4	11317.6	13165.9	12893.5	12553.6	12928
	5	14183	13734	12026.9	13791.1	12852
Average		13014.5	12885.7	11940.8	12380.7	12260.2
SUN-	1	113212	12254.5	12807.4	11407.4	11216.6
FLOWER	2	13862.8	13136	12274.5	12770.4	12195
PALM OIL	3	12968	12491.3	13323.3	12589.2	12755.4
MIX DIET	4	12787.4	12031	12747.4	13235	11402.3
Average		13207.6	12478.2	12788.2	12500.5	11892.3

Table II: Thromboxane B_2 radioimmunoassay counts per minute by diet

		WEEKS OF DIET				
		0	1	3	5	6
	1	13943.7	-------	7538.3	9719.5	12795.4
	2	14735	10953	8716.1	13684.1	8608
BUTTER	3	16096.2	9366	-------	13289	12562.1
DIET	4	13001.6	-------	7308.6	12464	12241.7
	5	10801.8	11364	9190	-------	12844.5
Average		13715.7	10561	8188.3	12289.2	11810.3
CRUDE	1	14936.2	10816.6	8223.5	9067.6	11658.5
PALM	2	14244.7	10450.4	8466	12852.4	11747.1
OIL	3	13014.9	8098.6	8519.9	13167	13249
DIET	4	14633.1	8778.3	8446.6	8857.9	13031
Average		14207.2	9536.0	8414	10986.2	12421.4
REFINED	1	8363.7	9980.6	8080.3	10438	12975.1
PALM	2	12680.3	9596.1	-------	8532.9	10968.6
OIL	3	14459	11337.9	10524	9752.5	11691.5
DIET	4	11906	11469.7	10236.7	15134.5	14095.1
Average		11852.3	10596.1	9613.7	10964.5	12432.6
SUN-	1	13876	9887.4	10053	13272.9	11395.4
FLOWER	2	15392	9557.2	9116.1	13696.7	14820.3
OIL	3	10059.9	9310	5962	11991.2	9847.6
DIET	4	-------	10056	8739.5	12343	-------
	5	15112	10426.9	11050.2	10122	7280.1
Average		13610.0	9847.5	8984.2	12285.2	10835.9
	1	10998.1	9405	8704	11492.1	12054.5
	2	9691	9262.1	9016.2	8404.7	12990.7
PARKAY	3	14142	11914.7	9727	-------	-------
DIET	4	14721.9	8480.3	8308.9	12757.7	13185
	5	10865.9	9974	9479.5	12794.8	12412
Average		11569.2	9807.2	9047.1	11362.3	12660.6
SUN-	1	14639.3	8615	7675.8	11462.5	13263
FLOWER	2	10587.6	10802	941903	12122.4	12127
PALM OIL	3	15181.2	9755	8639.9	7978.8	13589.6
MIX DIET	4	13529.3	9058	9412.1	14584	11809.6
Average		12404.8	9557.5	8787.7	11536.9	12697.3

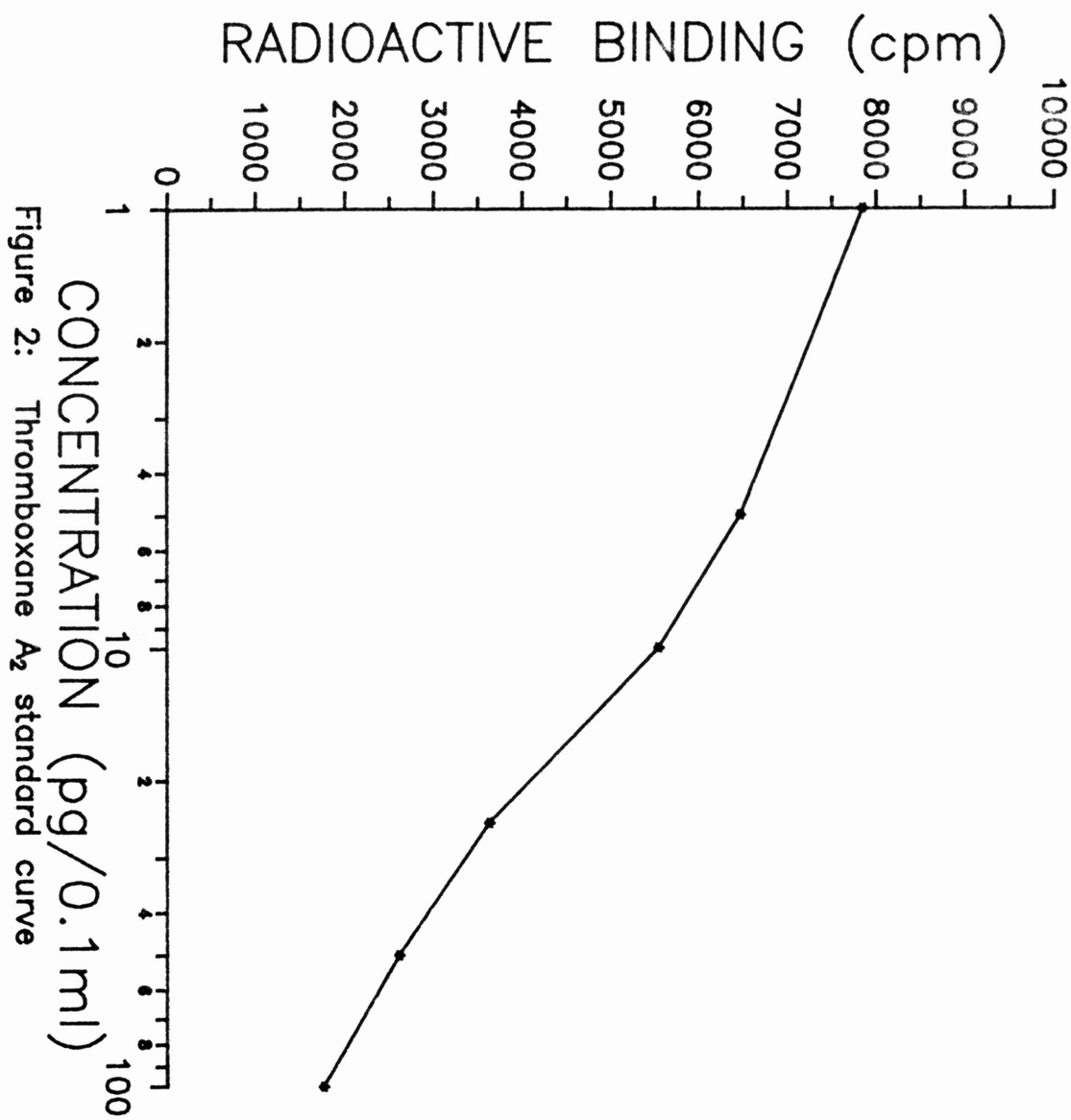


Figure 2: Thromboxane A_2 standard curve

decrease in counts per minute, indicating a probable increase in the sample thromboxane B_2 present, while the refined palm oil and margarine diets showed an increase in counts per minute, indicating a decrease in the thromboxane B_2 present. These results are shown in Figure 3 and Figure 4.

In the protaglandin assay, the butter, crude palm oil, and refined palm oil diets showed very little change from baseline to week six of the diet (Table II). There was some variation in weeks one, three, and five, but overall, the average counts per minutes were virtually the same in weeks zero and six (Figure 5). The sunflower oil, margarine, and sunflower/palm oil mix diets showed a downward trend, with only slight variablity, in average radioactive binding (Figure 6). This would indicate an increase in the levels of prostaglandin in the blood.

DISCUSSION

The results of this experiment cannot be used directly because the sample radioactive counts could not be plotted on a standard curve, probably due to non-specific binding. Even though a trend in the prostaglandin assay of the sunflower oil, margarine, and sunflower/palm oil mix diets was present, as well as changes in all the thromboxane assays, the decrease in average counts per minute might not have been due

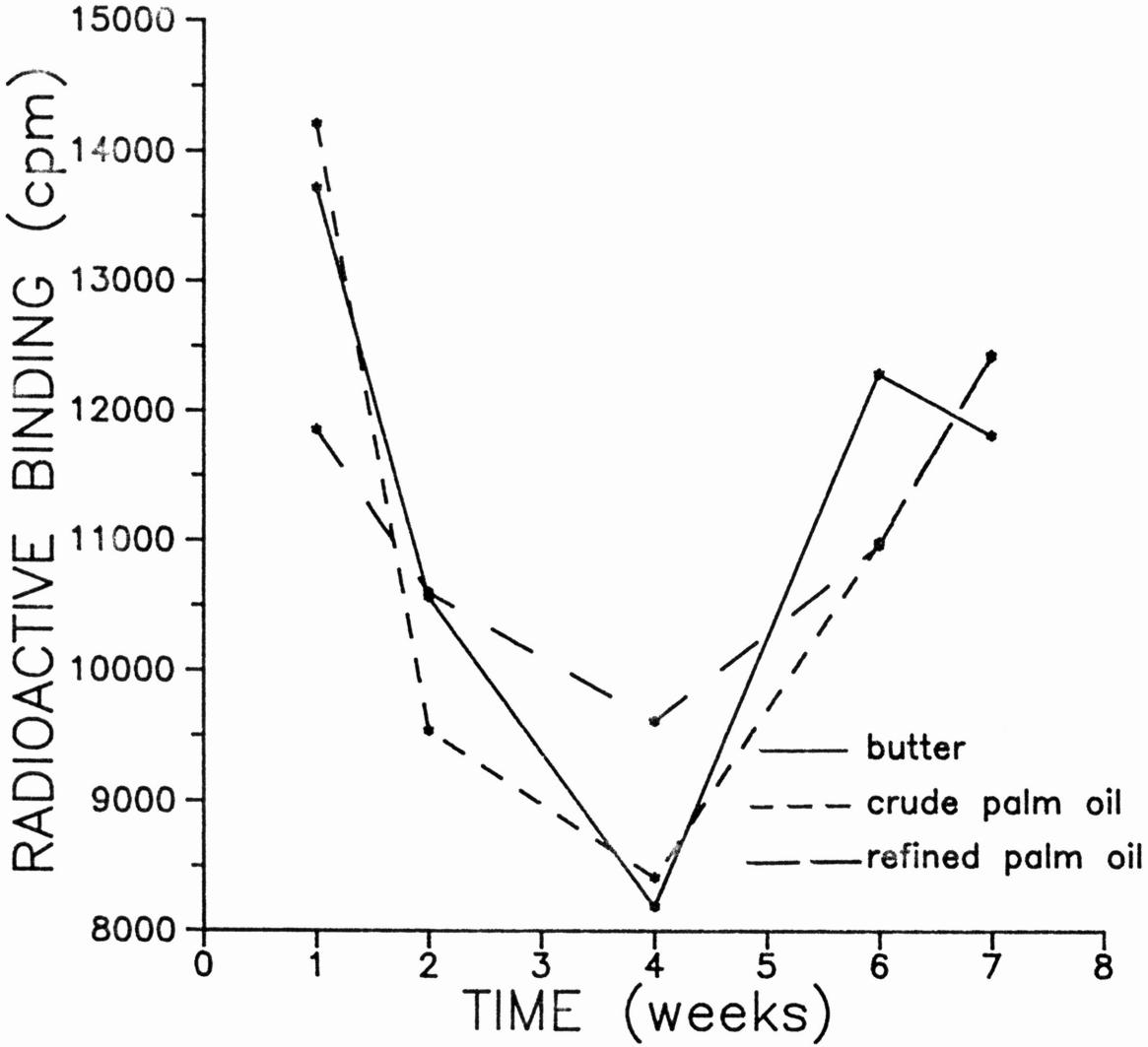


Figure 3: Influence of butter, crude palm oil, and refined palm oil on plasma thromboxane A_2 levels

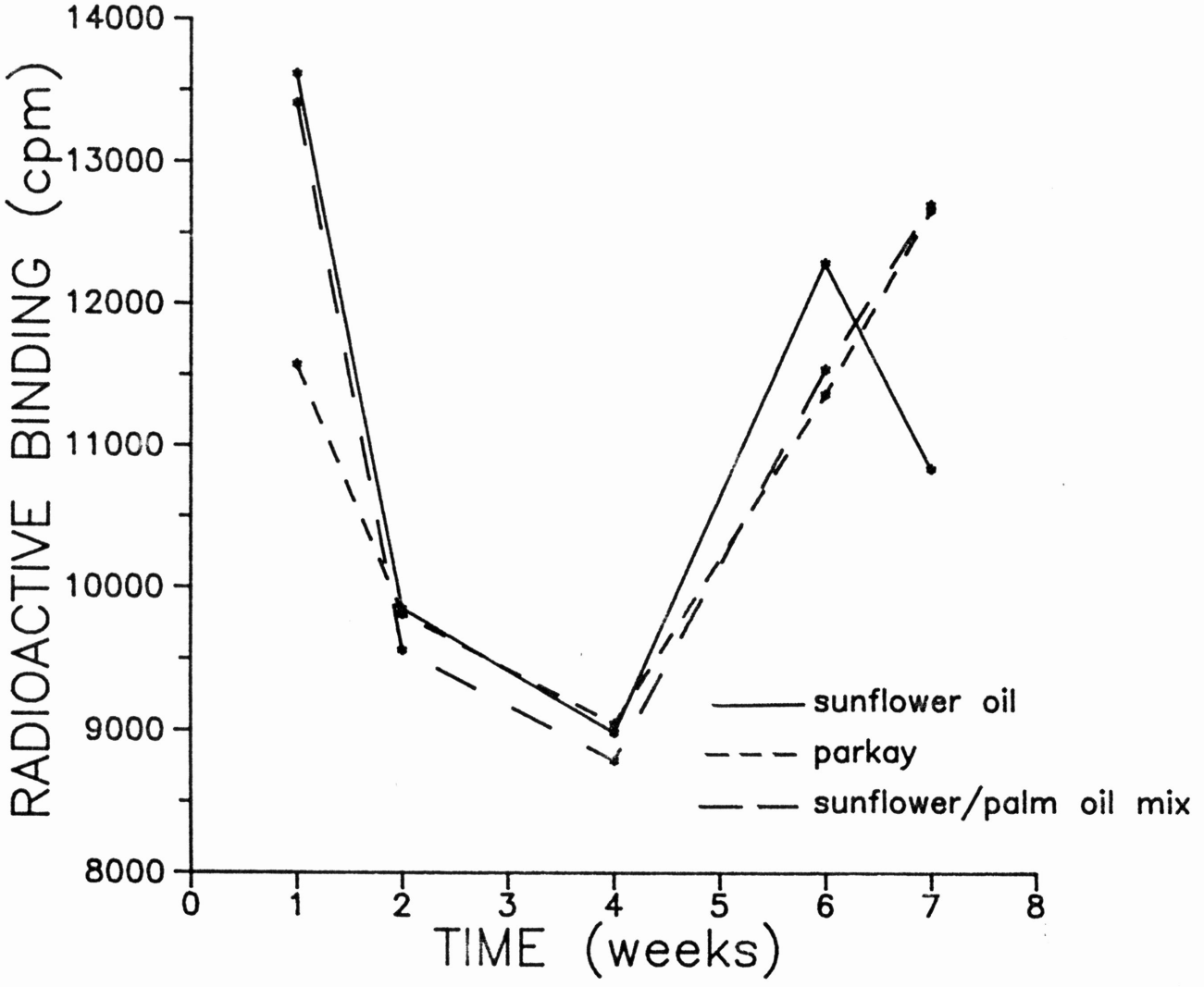


Figure 4: Influence of sunflower oil, parkay, and sunflower/palm oil mix on plasma thromboxane A_2 levels

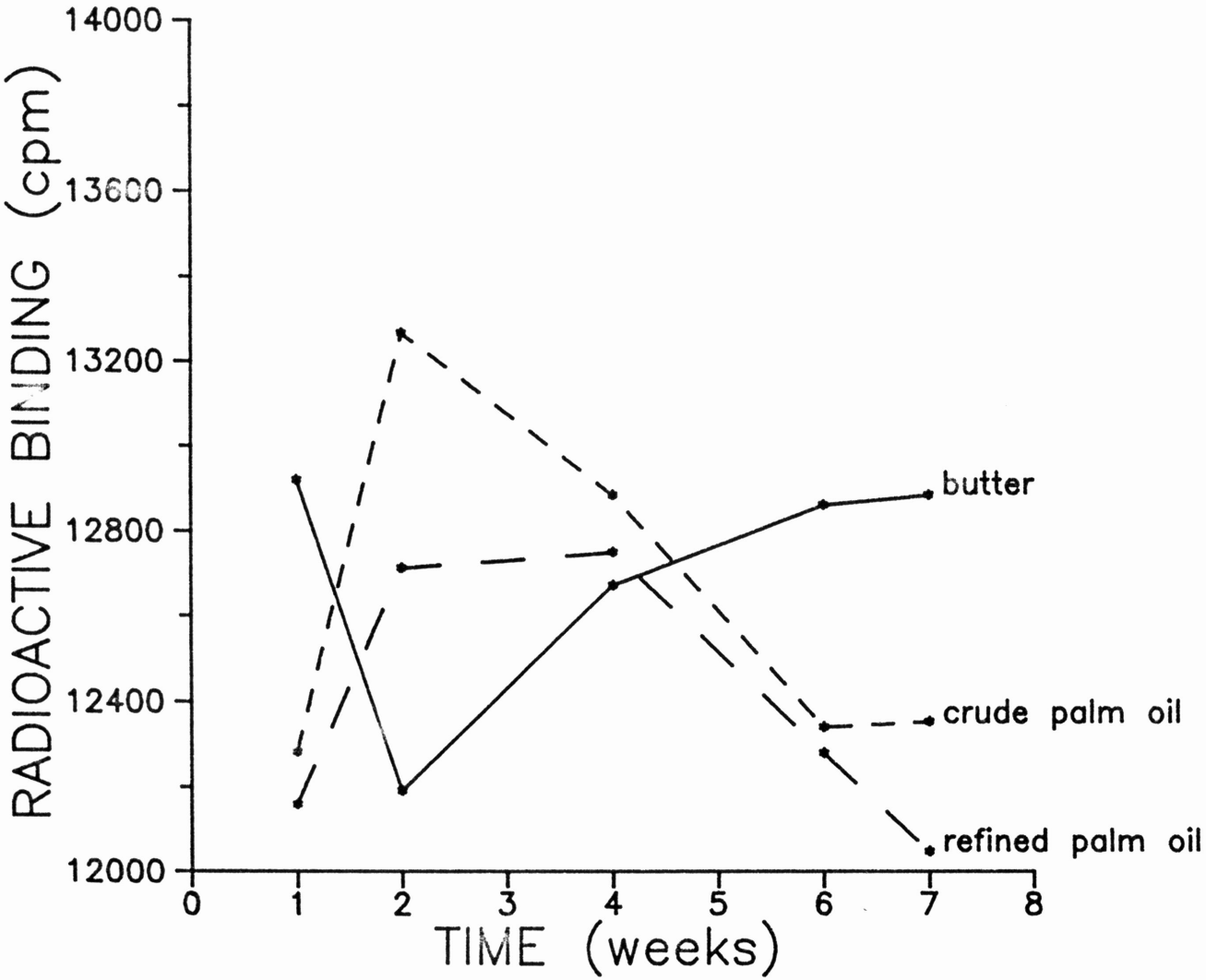


Figure 5: Influence of butter, crude palm oil, and refined palm oil on plasma prostacyclin levels

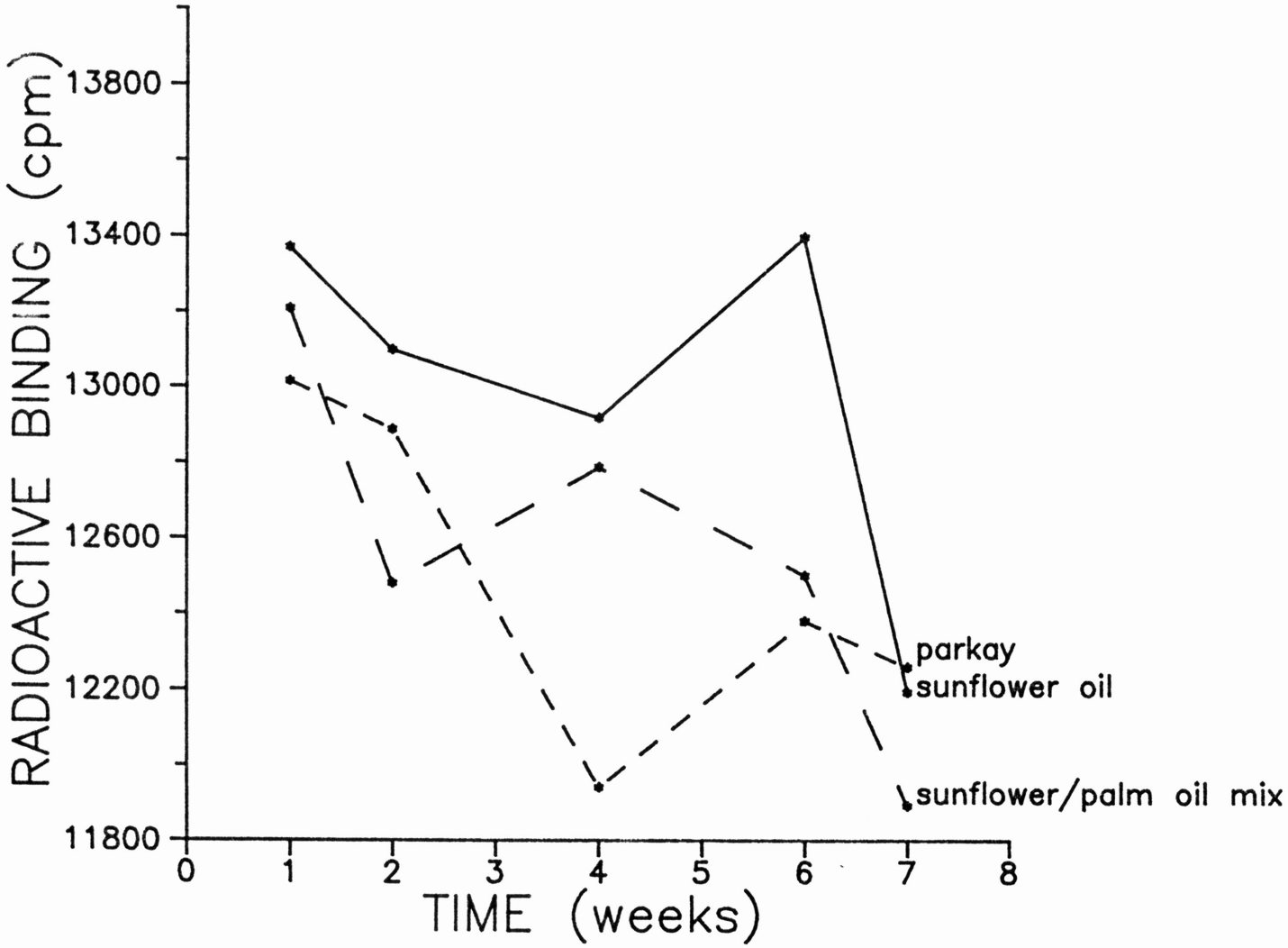


Figure 6: Influence of sunflower oil, parkay, and sunflower/ palm oil mix on plasma prostacyclin levels

to an increase or decrease in prostacyclin or thromboxane A_2 levels, but rather to a change in the assumed element in the plasma which is causing the non-specific binding. The large variability, especially in the thromboxane B_2 assay, in the radioactive binding seems to indicate that the non-specific binding element is variable in all the plasma samples. If this is true, any changes in average counts per minute could be due to a change in this element rather than a change in the compound of interest.

The results indicate that there is very little change in the concentrations of prostacyclin and thromboxane A_2 due to diet. If dramatic increases in the concentrations of either of these elements had occurred, the radioimmunoassays would have indicated it. The most that can be concluded from the results obtained is that none of the subjects had the abnormally high plasma levels of prostacyclin or thromboxane A_2 that can be seen in some heart patients. It appears that the human body is able to adjust to changes in diet without major changes in the levels of these compounds.

Because the results were inconclusive, however, the study should be continued to determine whether or not prostacyclin and thromboxane A_2 levels in the blood change with changes in dietary lipid intake.

FUTURE RESEARCH

This dietary study is scheduled to continue through five more diet periods. Each of the subjects will spend six weeks on each of the diets. Blood sample will continue to be collected for determination of prostacyclin and thromboxane A_2. This time, however, unless a means of eliminating the non-specific binding is found, a different assay method will be used. Mass spectroscopy is a sensitive assay method which could be used to measure these compounds. It is extremely specific as it is based on the structure of the compound (12). Also, high performance liquid chromatography could be used as an assay method. It is less sensitive than mass spectroscopy, but it is able to analyze serum without any additional sample preparation, unlike mass spectroscopy. Using these assay methods, perhaps the hypothesis of this study can be proven or disproven.

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