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Todd, Mikel Kent, Ph.D.

The University of North Carolina at Greensboro, 1990

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EFFECT OF AGE AND THIRTY MINUTES OF EXERCISE ON PROSTACYCLIN/THROMBOXANE A_2 RATIOS AND CIRCULATING CONCENTRATIONS OF PROSTACYCLIN AND THROMBOXANE A_2

by

Mikel Kent Todd

A Dissertation Submitted to
the Faculty of the Graduate School at
The University of North Carolina at Greensboro
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1990

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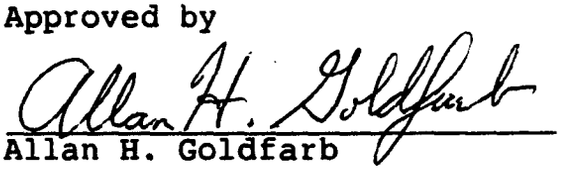

Allan H. Goldfarb

APPROVAL PAGE

This dissertation has been approved by the following committee of the Faculty of the Graduate School at The University of North Carolina at Greensboro.

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The effect thirty minutes of treadmill exercise on prostacyclin/thromboxane A_2 (PGI_2/TXA_2) ratios, plasma PGI_2 and TXA_2 in young and older men, 27.8 $\pm$ 0.8 and 55.4 $\pm$ 1.3 years old, respectively, was determined. Exercise intensity was maintained at 70-75% of VO_2max. Venipuncture samples were taken at rest, immediately after exercise, and at thirty minutes recovery. Total cholesterol (TC), high density lipoprotein (HDL), and low density lipoprotein (LDL) were determined from a serum sample taken at rest. Plasma 6-keto-prostaglandin $F_{1\alpha}$ and TXB_2 (i.e. stable metabolites of PGI_2 and TXA_2, respectively) were determined by radioimmunoassay. Plasma PGI_2 and TXA_2 were corrected for hemoconcentration; TXA_2 was corrected for platelet count. Linoleic acid (LLA) intake and polyunsaturated/saturated fat ratios (P/S ratios) were estimated by four-day diet records.

Repeated measures ANOVA indicated that there was a significant main effect for age on PGI_2/TXA_2 ratios and TXA_2 concentrations. Resting PGI_2/TXA_2 ratios were 7.4 $\pm$ 1.4 for the young men and 5.9 $\pm$ 1.0 for the older men; the ratios were not significantly different. Immediate-post exercise ratios changed 25.4% and 10.3% for the young and older men, respectively. The difference was not significant. Thirty minutes after exercise PGI_2/TXA_2 ratios for the older men were 37.5% below resting levels, compared to an 8.2% decline

for the young men. There was an 85.2% difference between group ratios thirty minutes after exercise; the difference was not significant. Ratio changes for the older men thirty minutes after exercise were marked by a 28.7% increase in TXA_2 above resting values. Seperately adding TC, HDL, LDL, LLA and P/S ratios as covariates did not alter the analysis.

These data suggest that older men may experience declines in PGI_2/TXA_2 below resting values thirty minutes following exercise at 70-75% of VO_2max. Thus, older men, when compared to younger men, may be more predisposed to platelet aggregation thirty minutes after exercise.

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CHAPTER I

INTRODUCTION

Statement of the Problem

Clinical manifestations of coronary heart disease include angina, myocardial infarction and sudden death. Researchers report that these and other ischemic events may be initiated by platelet aggregation resulting from platelet contact with damaged endothelial tissue (Davies, & Thomas, 1984; Freeman, Williams, Chisholm, & Armstrong, 1989).

During the past decade much attention has been focused on the role of two prostanoids, thromboxane A_2 (TXA_2) and prostacyclin (PGI_2), in regulating platelet aggregation. Thromboxane A_2 is released from platelets and stimulates platelet aggregation; PGI_2 is released from endothelial tissue and inhibits platelet aggregation. Thromboxane A_2 is released when platelets come in contact with damaged endothelial tissue. This response may serve to protect the circulation by limiting blood loss and facilitating tissue repair. Prostacyclin, when released by the endothelium works to inhibit platelet aggregation. However, if the endothelial lining is sufficiently damaged, as in the presence of atherosclerotic plaque, PGI_2 release into the circulation may be too limited to inhibit platelet aggregation. Under these conditions, the platelet clump may become large enough to

impede blood flow and the clinical manifestations of myocardial ischemia may result.

Monocada and Vane (1979) suggested that the ratio between circulating concentrations of PGI_2 and TXA_2 has an important role in determining the extent of platelet aggregation; and, the lower the PGI_2/TXA_2 ratio the greater the predisposition towards platelet aggregation. Researchers have typically reported PGI_2 and TXA_2 concentrations separately, not as ratios. Thus, there is insufficient data available to determine what is normal for PGI_2/TXA_2 ratios.

Factors reported to independently influence circulating concentrations of PGI_2 and TXA_2 include: age, exercise and diet. Extensive research pertaining to the effect of age on in vitro prostanoid synthesis has been conducted. Vascular tissues removed from young laboratory animals have been found to release greater amounts of PGI_2 than vascular tissue extracted from older animals (Kent, Kitchell, Shand & Whorton, 1981; Chang & Tai, 1983). Giani et al. (1985) reported that platelets from younger rats synthesized less TXA_2 than platelets from older rats. These findings suggest that older animals may be more predisposed to platelet aggregation than younger animals.

The effect of age on in vivo concentrations of PGI_2 and TXA_2 in humans is less clear. Researchers report that circulating concentrations of both PGI_2 and TXA_2 increase with age; however, they do not indicate how these changes

affect PGI_2/TXA_2 ratios (Reilly & FitzGerald, 1986; Vericel, Croset, Sedivy, Courpron, Dechavanne & LaGarde, 1988). A review of the mean data from these studies suggests that PGI_2/TXA_2 ratios are either unaffected (Reilly & FitzGerald, 1986) or decline as age increases (Vericel, Croset, Sedivy, Courpron, Dechavanne & LaGarde, 1988). Further investigation of the effect of age on PGI_2/TXA_2 ratios in humans is warranted.

Exercise has been associated with increases in circulating concentrations of PGI_2 (Demers, Harrison, Halbert & Santen; 1981; Piret et al, 1990) and TXA_2 (Laustiola, Seppala, Nikkari & Vapaatalo, 1984; Taniguchi, Furui, Yamauchi & Sotobata, 1984). Other researchers report no change (Viinikka, Vuori & Ylikorkala, 1984) or a decline in PGI_2 (Taniguchi, Furui, Yamauchi & Sotobata, 1984), and no change in TXA_2 (Metha, Metha & Hovalek, 1983; Viinikka, Vuori & Ylikorkala, 1984) in response to exercise. Rauramaa (1987) suggested that inconsistencies among these studies may be due to different modes, intensities and durations of exercise used to stimulate prostanoid changes. Also, some of these studies involved subjects representing a wide range of ages (Demers, Harrison, Halbert & Santen, 1981; Metha, Metha & Horalek, 1983) or primarily young subjects (Piret et al., 1990). Research comparing the effects of exercise on PGI_2 and TXA_2 among or between different age groups is needed

since age may influence circulating concentrations of PGI_2 and TXA_2.

The dietary precursor for both PGI_2 and TXA_2 is linoleic acid, which is found in most vegetable oils. Linoleic acid intake within the range of six to ten percent of total energy intake is desirable for proper PGI_2 and TXA_2 synthesis (Dupont, 1987). Variations in fatty acid intake may lead to differences in PGI_2 and TXA_2 synthesis. Therefore, assessment of dietary intake of linoleic acid should provide valuable information to anyone investigating the effects of age and exercise on circulating concentrations of these prostanoids.

The influence of chronic exercise (i.e. aerobic exercise at least twenty minutes per day, three days per week) and diet on prostanoid synthesis may be partially mediated by their effect on plasma lipoproteins (Leon, 1988). High density lipoproteins (HDL) have been reported to be positively associated with PGI_2 synthesis; whereas, low density lipoproteins (LDL) have been reported to be inversely associated with PGI_2 production (Beitz & Forster, 1980). Therefore, assessment of the influence of serum lipoprotein concentrations may provide insight into differences in plasma PGI_2 and TXA_2 due to age or acute exercise.

Age, exercise, and diet may have an indirect role in platelet aggregation as a consequence of their influence on PGI_2 and TXA_2. Elucidation of the combined effects of age,

exercise and diet on PGI_2 and TXA_2 may further clarify why older adults are at higher risk of ischemic events associated with exercise.

Purpose

The purpose of this study was to evaluate whether or not there is a statistically significant difference between the circulating PGI_2/TXA_2 ratio in young adult males (25 and 35 years old) and older adult males (50 and 65 years old) during rest, exercise and recovery from exercise. Individual concentrations of both PGI_2 and TXA_2 were reported to help clarify the interpretation of any changes observed in the PGI_2/TXA_2 ratio. Also, since dietary intake of linoleic acid, the polyunsaturated to saturated fat ratio (P/S ratio), HDL and LDL are known to affect PGI_2 and TXA_2 these factors were examined.

Hypotheses

The researcher hypothesized that upon comparison of the two age groups:

1. There would be no significant difference between maximal oxygen consumption (VO_2max), highest heart rate achieved during the maximal exercise test, and the percent oxygen consumption ($\%VO_2$) during sub-maximal exercise.
2. There would be no significant difference between PGI_2/TXA_2 ratios, plasma PGI_2, or plasma TXA_2 during rest, exercise, or recovery.

3. There would be no significant difference between PGI_2/TXA_2 ratio, plasma PGI_2, or plasma TXA_2 during rest, exercise, or recovery when total serum cholesterol was used as a covariate.
4. There would be no significant difference between PGI_2/TXA_2 ratios, plasma PGI_2, or plasma TXA_2 during rest, exercise, or recovery when serum HDL was used as a covariate.
5. There would be no significant difference between PGI_2/TXA_2 ratios, plasma PGI_2, or plasma TXA_2 during rest, exercise, or recovery when serum concentration of LDL was used as a covariate.
6. There would be no significant difference between PGI_2/TXA_2 ratios, plasma PGI_2, or plasma TXA_2 during rest, exercise, or recovery when dietary linoleic acid was used as a covariate.
7. There would be no significant difference between PGI_2/TXA_2 ratios, plasma PGI_2, or plasma TXA_2 during rest, exercise, or recovery when the P/S ratio was used as a covariate.

Assumptions and Limitations

Each subject was asked not to take medications known to inhibit prostanoid synthesis during this study. The researcher reminded the subjects about this instruction during the maximal exercise test session. Also, the researcher asked each subject during the sub-maximal exercise

session when he had last taken any medications known to inhibit prostanoid synthesis. Otherwise the researcher can only assume that this instruction was followed. Failure of subject compliance to this instruction may have lead to erroneous results.

Generalization of the results are limited by several factors. Only subjects determined to be at no or limited risk for coronary heart disease participated, as described in the methods chapter. No subjects displaying or reporting symptoms of coronary heart disease participated. The researcher attempted to recruit physically active subjects; and, subjects were self-selected.

Each subject exercised at the same relative intensity; but, the absolute workloads were different. The researcher did not know, a priori, if the lower absolute workload in the older group would be sufficient to alter prostanoid concentrations.

Dietary intake of linoleic acid, polyunsaturated fat and saturated fat were evaluated from dietary food records. The circulating concentrations of these substances were not determined. Consequently, the influence of these dietary substances on the circulating concentrations of PGI_2 and TXA_2 can only be regarded as statistical estimates.

Fann et al. (1989) have found regional variability in the synthesis of PGI_2 among different vessels extracted from laboratory animals. In the present study circulating

concentrations of PGI_2 were measured in blood taken from an antecubital vein. Therefore, the concentrations of prostacyclin found in the present study should not be considered representative of concentrations existing in parts of the circulation other than antecubital venous areas.

CHAPTER II

REVIEW OF RELATED LITERATURE

Prostanoid Structure and Synthesis

In the early 1930's von Euler and Goldblatt independently discovered prostanoids. Von Euler used the term "prostaglandin" to describe a substance in human semen capable of stimulating smooth muscle contraction and lowering blood pressure. The discovery of prostanoids was largely ignored until the 1960's when researchers at the Karolinska Institute in Stockholm determined the chemical structure of the first prostanoid (Cohen, 1985).

Prostanoids can be synthesized from three different 20-carbon unsaturated fatty acids: eicosatrienoic acid, eicosatetraenoic acid (arachidonic acid) and eicosapentaenoic acid. These fatty acids vary in degree of unsaturation. Eicosatrienoic acid has three double bonds, arachidonic acid has four and eicosapentaenoic acid has five. The prostanoids synthesized from these substrates are given series designations A through I and a subscript numbers of 1, 2 or 3. The letter designation denotes the prostanoid's chemical configuration. The subscript number denotes the number of double bonds present in the prostanoid's two side chains. Sometimes the Greek letter alpha or beta follows the subscript number. This letter denotes the stereochemistry

of the prostanoid at the C9 position. Whereas prostanoids retain their 20-carbon backbone indicative of their fatty acid substrates, the structure of the side chains is altered. The enzymes responsible for synthesizing prostanoids are present in most cells and synthesis is apparently stimulated by slight alterations in tissue homeostasis (Cohen, 1985).

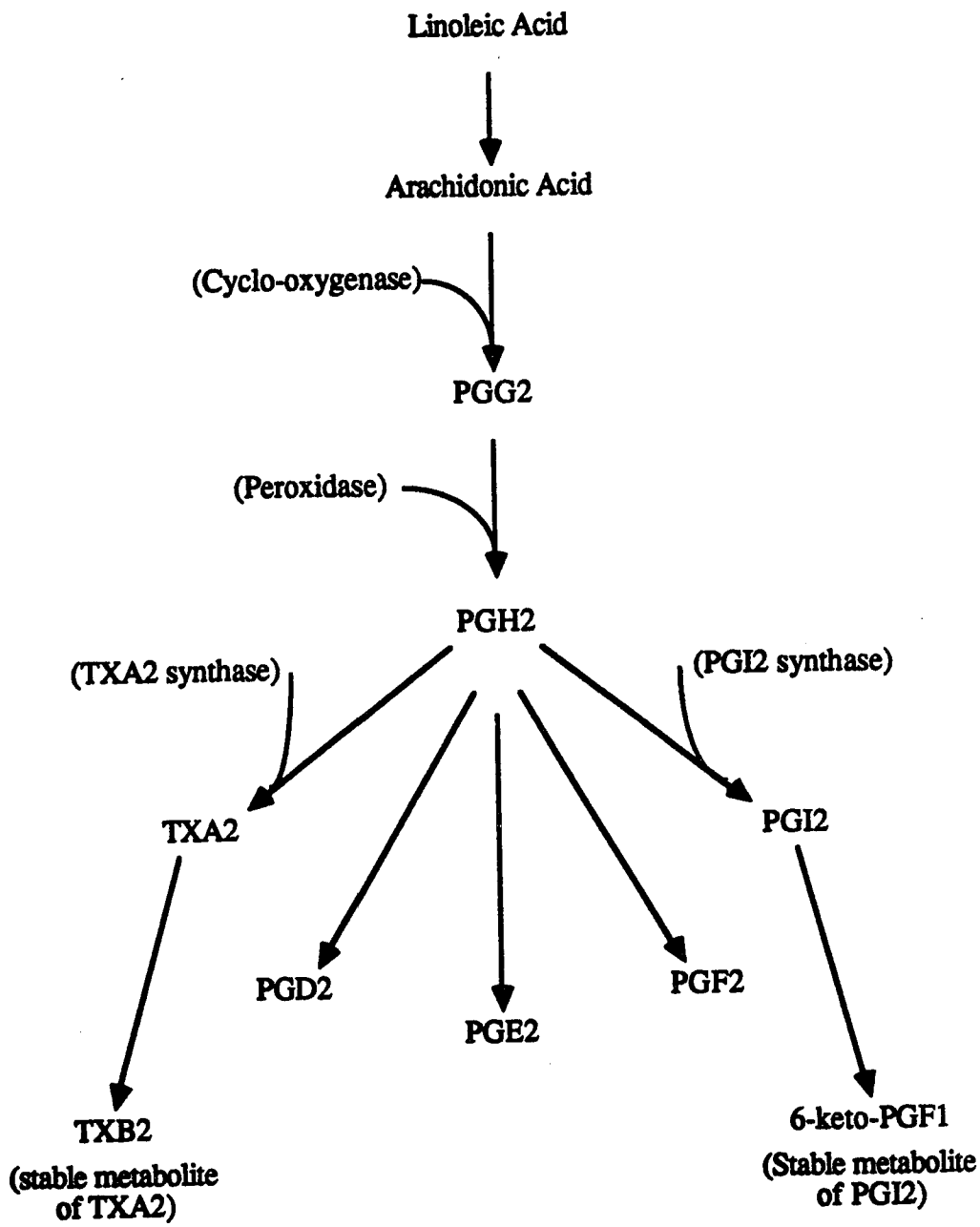
PGI_2 and TXA_2 Structure and Synthesis

In 1976 researchers discovered that prostaglandin endoperoxides were changed by endothelial enzymes into an substances with potent anti-aggregatory properties. They later found that this substance relaxed vascular strips in vitro, while in vivo it dilated vascular beds and had antithrombotic properties. Originally named PGX, it was renamed prostacyclin and given the abbreviation PGI_2 when it's chemical structure was determined by the Wellcome Research Laboratory and the Upjohn Company (Cohen, 1985).

The chemical structure of TXA_2 was determined near the same time as PGI_2. Since TXA_2 was first found in platelets and determined to promote platelet aggregation it was given the name "thrombo"xane. In opposition to PGI_2, TXA_2 has potent aggregatory properties. Thus, the PGI_2/TXA_2 ratio is believed to have a profound influence on platelet aggregation.

Arachidonic acid (AA) is the precursor for both PGI_2 and TXA_2 (Figure 1). Arachidonic acid may be supplied directly by the diet or synthesized from the 18-carbon linoleic acid

Figure 1. Prostaglandin Metabolism



which is present in most vegetable oils. The first steps in PGI_2 and TXA_2 synthesis are catalyzed by cyclooxygenase and peroxidase. Cyclooxygenase is responsible for the cyclization and oxygenation of AA into the unstable intermediate prostaglandin G_2 (PGG_2). Peroxidase facilitates the change of PGG_2 into prostaglandin H_2 (PGH_2), which is also an unstable intermediate. Prostaglandin G_2 and PGH_2 are frequently referred to as cyclic endoperoxides (Cohen, 1985).

Several tissue enzymes are capable of acting upon PGH_2. Prostaglandin I_2 synthetase changes PGH_2 into PGI_2 and TXA_2 synthetase converts PGH_2 to TXA_2, respectively. Prostaglandin I_2 synthetase is present in blood vessel walls, with the greatest portion found in the endothelium. Thromboxane A_2 synthetase is primarily found on platelet surfaces (Cohen, 1985).

Influence of Age upon PGI_2 and TXA_2

Results from several studies involving animal models indicate that PGI_2 synthesis is inversely associated with age; whereas, TXA_2 synthesis remains unchanged or increases with age. An outline the studies investigating the effect of age on PGI_2 and TXA_2 in animals is presented in Table 1.

Chang and Tai (1983) reported that aortic rings removed from twelve-month-old rats produced significantly more PGI_2 (14.0 ± 0.78 ng/mg dry weight) than aortic rings from twenty-four month old rats (8.8 ± 0.76 ng/mg dry weight). No age related difference in TXA_2 synthesis was found; but, in a

Table 1.

Age and Prostanoids in Animals

Researchers	Date	Research model	Treatment	PGI_2	TXA_2
Chang et al.	1980	aortic cells from 12 & 24 month old male Fischer rats	30 min incubation in arachidonic acid (AA)	PGI_2/PGE_2 ratio 12 m: 2.40 ± .05 24 m: 0.67 ± .06	N.A.
Chang & Tai	1983	aortic cells and platelets from 2, 12 & 24 month old male Fischer rats	aortic cells: 30 min incubation in AA; platelets: 2 min incubation in AA	(ng/mg dry wt) 2 m: 7.2 ± .8 12 m: 14.0 ± .8 24 m: 8.8 ± .8	(ng/mg dry wt) 2 m: 85 ± 5.9 12 m: 34 ± 6.5 24 m: 117 ± 10.6
Giani et al.	1985	aortic cells and platelets from 1& 11 month old Sprague Dawley rats	aortic cells: perfused with plasma; platelets: 5 min incubation in collagen	perfused (pg/ul) 1 m: 6.6 ± 0.8 11 m: 14.6 ± 2.4	(pg/ul) 1 m: 43.6 ± 2.9 11 m: 85.9 ± 10.8
Kent et al.	1981	aortic cells from 6 month and 2-4 year old swine	30 min incubation in AA	(ng/gm of tissue) 6 m: 3299 ± 437 2-4 y: 1706 ± 252	N.A.
Menconi et al.	1987	aortic cells from neonatal 13 & 30 month old Sprague Dawley rats	20 min incubation in bradykinin or ionophore A23187	PGI_2 vs PGE_2 neo.: $PGI_2 > PGE_2$ 13m: $PGI_2 = PGE_2$ 30m: $PGI_2 < PGE_2$	N.A.
		pulmonary artery; tissue cultures from swine; 12 & 108 population doublings (PD)	30 min incubation in AA	(ng/ml) 12 PD: 132.0 ± 12.0 108 PD: 1.8 ± 0.2	N.A
		adventitial fibroblasts from swine; 13 & 65 PD	30 min incubation in AA	(ng/ml) 13 PD: 1.3 ± 0.1 65 PD: 0.5 ± 0.1	N.A.
Panganamala et al.	1981	aortic cells from 3 to 20 week old Sprague Dawley rats	30 min incubation in AA	(pm/ng of tissue) 3 w: @ 17 12 w: @ 32 20 w: @ 60	N.A.

N.A. = not available
w = week
m = month

similar study Giani, Masi and Galli (1985) found that platelets taken from eleven month old rats produced significantly more TXA_2 (85.9 $\pm$ 10.8 pg/ul) than platelets from one month old rats (43.6 $\pm$ 2.9 pg/ul). Kent et al. (1981) reported significantly greater PGI_2 production in aortic tissue from six month old swine (3299 $\pm$ 437 ng/gm) when compared with two to four year old swine (1706 $\pm$ 252 ng/gm). The differences found by Kent et al. (1981) existed only when arachidonic acid was present in high concentrations. Thromboxane A_2 was not measured in this study.

The influence of age upon prostanoid synthesis has also been studied in vitro. Chang et al. (1980) found that while total prostanoid synthesis was similar between cell cultures from twelve and twenty-four month old rats, significantly more PGI_2 was synthesized in the cultures from the twelve month old rats. Cell cultures from the twenty-four month old rats produced greater quantities of prostaglandin E_2 (PGE_2) than PGI_2. The PGI_2/PGE_2 ratios were reported as 2.4 $\pm$ 0.05 and 0.67 $\pm$ 0.062 for the young and old rats, respectively. In the same study, Chang et al. (1980) bypassed several steps in the linoleic acid pathway by adding PGH_2 directly to the cell cultures from each group. The researchers reported that the cultures from the twelve month old rats converted six times as much of the PGH_2 to PGI_2 than the cultures from the twenty-four month old rats. These data suggest that the

enzyme responsible for conversion of PGH_2 to PGI_2 was less active in the older cultured cells. Chang et al. (1980) did not measure TXA_2.

Menconi et al. (1987), grew smooth muscle cells in culture from neonatal, thirteen month old and thirty month old rats. After twenty minutes exposure to bradykinin or ionophore A23187, both stimulants of PGI_2 synthesis, prostanoid products were analyzed. Prostaglandin I_2 synthesis was highest in the neonatal rat cultures and lowest in the thirty month old rat cultures. Menconi et al. (1987) also investigated PGI_2 production in the microvasculature and the relationship of such production to age. The researchers reported that PGI_2 production decreased with age. Menconi et al. (1987) did not provide quantitative results.

Tissue cultures of human skin fibroblast represent another research model that investigators have used to examine the relationship of PGI_2 synthesis to age. This research model is useful because human skin fibroblast are known to have doubling capacities limited by age. Taylor et al. (1981) found that tissue cultures which underwent a greater number of doublings (i.e. older cultures) synthesized significantly less PGI_2 than cultures which underwent fewer doublings (i.e. younger cultures). In this study tissue cultures were stimulated separately with AA, ascorbic acid, and bradykinin. In each case, PGI_2 concentrations after stimulation were lower in the older cultures. In a similar

study, Polgar and Taylor (1980) found that younger human embryo lung fibroblast cultures synthesized a greater quantity of PGI_2 (0.92 $\pm$ 0.08 ng/ml) when compared to older cultures (0.53 $\pm$ 0.03 ng/ml). In addition, the older cultures synthesized greater amounts of TXA_2 and PGE_2; while the oldest cultures synthesized predominantly prostaglandin $F_{1\alpha}$ ($PGF_{1\alpha}$). Chan et al. (1975) suggested that $PGF_{1\alpha}$ can be synthesized non-enzymatically. Thus, the findings by Chan et al. (1975) and Polgar and Taylor (1980) support the theory that the influence of aging upon the production of PGI_2 may be mediated by a reduction in PGI_2 synthetase activity.

Only a few studies involving the investigation of PGI_2 and TXA_2 concentrations in humans have been conducted. These studies are outlined in Table 2.

Hanley and May (1985) investigated PGI_2 synthesis by saphenous venous tissue removed from patients undergoing varicose vein surgery. These investigators found no relationship between PGI_2 synthesis and age. The results of this investigation, however, may have been confounded by the fact that many of the patients were smokers. Several researchers have reported that smoking may inhibit PGI_2 synthesis (Pittilo, Mackie, Rowles, Machin & Woolf, 1982; Sonnenfeld & Wennmalm, 1980; Wennmalm, 1980).

The results from studies investigating in vivo concentrations of PGI_2 and TXA_2 in apparently healthy humans are not consistent. Although researchers have reported that

Table 2.

Age and Prostanoids in Healthy Humans

Researchers	Date	Subjects	Treatment	PGI_2	TXA_2
Hanley & May	1985	saphenous venous tissue from 51 men and 117 women having varicose vein removal; 17 to 70 years old	30 min incubation in buffer (smokers included)	No significant relationship between age and PGI_2 concentrations	N.A.
Reilly & FitzGerald	1986	12 (8 males + 4 females) 21 - 39 years old (Y); 20 (10 males + 10 females) 50 - 88 years old (O)	resting; urinary PGI_2 and TXA_2	(pg/mg creatinine) Y: 121 ± 13 O: 197 ± 21	(pg/mg creatinine) Y: 152 ± 19 O: 223 ± 22
			resting; serum TXA_2		(ng/ml) Y: 367 ± 33 O: 327 ± 43
Vericel et al.	1988	8 (sex not given), 25 - 35 year olds (Y); 16 (sex not given), 78 - 94 year olds	resting; urinary PGI_2 and TXA_2	(ng/mole creatinine) Y: 116 ± 42 O: 193 ± 118	(ng/mole creatinine) Y: 25.1 ± 14.5 O: 51.5 ± 43.2
			resting; serum PGI_2 and TXA_2	(nmoles/ml) Y: 13.3 ± 8.1 O: 27.2 ± 14.8	(nmoles/ml) Y: 0.49 ± 0.18 O: 0.28 ± 0.19
			resting; TXA_2 stimulated with thrombin	N.A.	(nmoles/10^9 platelets) Y: 0.65 ± 0.22 O: 1.09 ± 0.52

N.A. = not available
Y = young subjects
O = older subjects

urinary concentrations of both PGI_2 and TXA_2 increase with age (Reilly & FitzGerald, 1986; Vericel, Croset, Sedivy, Courpron, Dechavanne, & LaGarde, 1988), the influence of these age related changes on the PGI_2/TXA_2 ratio vary. Based on the mean data reported by Reilly and FitzGerald (1986) the ratio was similar in both age groups; whereas, the data from the study by Vericel et. al. (1988) suggest that the ratio was lower in the older age group.

In summary, the results from research conducted in vitro and with tissue samples from animals suggest that PGI_2 synthesis is inversely associated with age, and that TXA_2 synthesis is unaffected by age. In vivo studies involving apparently healthy humans do not entirely support these findings, although there are some consistencies suggestive of an age related reduction in the PGI_2/TXA_2 ratio.

Exercise and Prostanoids

Only the studies involving the acute effects of exercise on prostanoids will be reviewed, although researchers have investigated the effects of both acute and chronic exercise on prostanoids. This review is also limited to studies involving apparently healthy subjects. An outline of the research investigating the effects of acute exercise on PGI_2 and TXA_2 in apparently healthy individuals is presented in Table 3.

In 1981, Demers et al. found that plasma PGI_2 was significantly higher in athletes immediately following a

Table 3.

Exercise and Prostanoids in Healthy Humans

Researchers	Date	Subjects	Treatment	PGI_2	TXA_2
Carter et al.	1989	12 male athletes; 23.7 ± 3.4 years old; VO_2max: 56.2 ± 5.7 ml/kg/min	15 min cycling at 30% of VO_2max; before exercise (BE); during exercise (DE); after exercise (AE)	(pg/min plasma) BE: 2.92 ± 0.62 DE: 6.16 ± 1.87 AE: 3.16 ± 0.59	(nmol/l plasma) BE: 6.40 ± 2.38 DE: 9.64 ± 3.45 AE: 13.01 ± 5.34
		9 male runners; 31 ± 6 years old	21 to 42 km run; before race (BR); immediately after race (IR)	(pg/min plasma) BR: 1.62 ± 0.30 IR: 1.08 ± 0.24	(nmol/l plasma) BR: 6.88 ± 1.24 IR: 8.48 ± 1.65
Demers et al.	1981	20 males; 40 ± 10 years; 4 females; 37 ± 7 years	42 km run	(pg/ml plasma) BR: 2161 ± 335 AR: 4462 ± 2405	N.A.
Laustiola et al.	1984	6 males; moderately trained; 19 - 23 years old	exhaustive exercise at 50% of "maximal aerobic capacity"; immediately after exercise (IE); 30 min after exercise (AE)	(% of resting value) IE: 40% above rest AE: 10% above rest	(pg/ml plasma) BE: @ 130 IE: @ 250 AE: @ 275
				N.A.	(pg/ml serum) BE: @ 40 IE: @ 50 AE: @ 75
Metha et al.	1983	13 males; 22 - 65 years old	maximal exercise test 10 min minumum; peak exercise (PE); 15 - 30 after exercise (AE)	(pg/ml plasma) BE: 54 ± 17 PE: 175 ± 57 AE: 61 ± 19	(pg/ml plasma) BE: 135 ± 30 PE: 168 ± 42 AE: 97 ± 22

N.A. = not available

Table 3. (continued)

Exercise and Prostanoids in Healthy Humans

Researchers	Date	Subjects	Treatment	PGI_2	TXA_2
Piret et al.	1990	10 males; 22.2 ± 0.1 years old; untrained	cycle exercise to exhaustion; 1 hour after exercise (AE)	(pg/mg creatinine) BE: 55 AE: 131	N.A.
Taniguchi et al.	1984	26 males; 21 - 41 years old;	treadmill exercise at 85% predicted maximal heart rate for 12 min; 30 min after exercise (AE)	(ng/ml plasma) BE: 1.12 ± 0.2 IE: 1.00 ± 0.17 AE: 1.34 ± 0.36	(pg/ml plasma) BE: 80 ± 17 IE: 118 ± 21 AE: 118 ± 27
Todd et al.	1990	6 males; 27.3 years old	treadmill exercise at 60%, 70% and 80% of VO_2max for 30 min	(pg/ml plasma) BE: 384 IE60: 512 IE70: 430 IE80: 389	(pg/ml plasma) BE: 147 IE60: 523 IE70: 612 IE80: 644
Viinikka et al.	1984	10 runners; (9 male + 1 female); 18 - 30 years old; 10 controls	cycle exercise to exhaustion; 7 min of exercise (DE); 30 min after exercise (AE)	(pmol/l plasma) BE: 192.8 ± 51.7 DE: 260.5 ± 86.9 IE: 214.3 ± 62.2 AE: 170.6 ± 45.7	(nmol/l serum) BE: 703.3 ± 290.1 DE: 810.8 ± 326.2 IE: 798.9 ± 206.1 AE: 727.3 ± 213.0

N.A. = not available

marathon run. Plasma PGI_2 was 2161 $\pm$ 335 pg/ml before exercise and 4462 $\pm$ 2405 pg/ml following exercise. These researchers did not measure TXA_2 levels. Viinikka et al. (1984) studied the effects of ten to fourteen minutes of exhaustive exercise on circulating prostanoids. They found that plasma PGI_2 concentrations in marathoners before (192.8 $\pm$ 51.7 pmol/L) and after (214.3 $\pm$ 62.2 pmol/L) stationary cycling were not significantly different from concentrations found in non-exercising controls (206.1 $\pm$ 75.7 pmol/L). At the seventh minute of exercise, however, plasma PGI_2 was significantly greater than resting values (260.5 $\pm$ 86.9 pmol/L). Thirty minutes following exercise plasma PGI_2 (170.6 $\pm$ 45.7 pmol/L) tended to be lower than pre-exercise concentrations. Viinikka et al. (1984) reported a fifteen percent increase in serum TXA_2 during exercise. This increase was not significant. In contrast, Laustiola et al., (1984) found that among well-trained distance runners plasma TXA_2 increased during exhaustive exercise on a cycle ergometer. A graphic display of the data indicated that resting plasma TXA_2 was approximately 135 pg/ml; at exhaustion plasma TXA_2 was approximately 260 pg/ml. Laustiola et al. (1984) also reported that plasma PGI_2 increased 40 percent when exercising subjects reached exhaustion.

In a study where subjects exercised on a treadmill at approximately 85 percent of their age predicted maximal heart

rate, Taniguchi et al. (1984), reported that plasma PGI_2 tended to decrease and plasma TXA_2 tended to increase. These changes were not significant. In a similar study, Metha et al. (1983) evaluated changes in plasma PGI_2 and TXA_2 in response to sub-maximal treadmill exercise at approximately 60% of the subjects' age predicted maximal heart rate. At rest PGI_2 and TXA_2 concentrations were 54 $\pm$ 17 pg/ml and 135 $\pm$ 30 pg/ml, respectively. These researchers reported that PGI_2 increased significantly during exercise (175 $\pm$ 57 pg/ml). Thromboxane A_2 tended to increase (168 $\pm$ 42 pg/ml), but the change was not significantly different from resting values.

Discrepancies in the results between studies using different exercise protocols prompted Todd et al. (1990) to investigate the effect of exercise intensity on circulating PGI_2 and TXA_2. Resting PGI_2 was 384 $\pm$ 68 pg/ml and TXA_2 was 147 $\pm$ 56 pg/ml. Exercise-induced alterations in plasma PGI_2 were not significantly affected by exercise intensity, although they tended to be inversely related to intensity. Plasma PGI_2 at 60, 70, and 80 percent of maximal oxygen consumption was 512 $\pm$ 52, 430 $\pm$ 55, and 389 $\pm$ 83 pg/ml, respectively. Increases in serum TXA_2 appeared to be positively associated with exercise intensity, although exercise did not have a statistically significant effect. Serum TXA_2 at 60, 70, and 80 percent of maximal oxygen consumption was 523 $\pm$ 117, 612 $\pm$ 155, and 644 $\pm$ 122 pg/ml,

respectively. Thirty minutes after exercise at 80 percent of maximal oxygen consumption TXA_2 (918 $\pm$ 188 pg/ml) was significantly greater than the pre-exercise concentration and the 60 (600 $\pm$ 138 pg/ml) and 70 (549 $\pm$ 132 pg/ml) percent concentrations observed at the same time. One weakness in the study conducted by Todd et al. (1990) was that blood was collected with the aid of an intravenous catheter and no data controlling for the effects of the catheter on prostanoid concentrations was collected.

Carter et al. (1989) conducted two separate studies intended to contrast the effects of different types of exercise on circulating concentrations of PGI_2 and TXA_2. These researchers found that during fifteen minutes of cycling at 30% of VO_2max subjects experienced a two-fold increase in plasma PGI_2 and a 50% increase in plasma TXA_2. Immediately following exercise PGI_2 returned to near resting concentrations and TXA_2 concentrations continued to rise to twice resting values. Only the two-fold increase in plasma TXA_2 immediately following exercise was significantly different from resting concentrations. Carter et al. (1989) also reported that following a 21 to 42 kilometer race both PGI_2 and TXA_2 concentrations were not significantly different than resting values. One weakness with the study by Carter et al. (1989) is that it was difficult to determine whether the type, intensity or duration of exercise was related to

the different results since each of these factors were different in the two studies.

Several researchers have suggested that the invasive nature of blood sampling may result changes in circulating prostanoids. This possibility has lead some researchers to investigate the effects of exercise on PGI_2 and TXA_2 by measuring urinary concentrations of these prostanoids. Wennmalm and FitzGerald (1988) reported that urinary PGI_2 was significantly greater than resting concentrations (74 $\pm$ 14 pg/mg creatinine) following two hours of exercise (267 $\pm$ 70 pg/mg creatinine) at 50 percent of the subjects' maximal oxygen consumption and following two hours of recovery (213 $\pm$ 35 pg/mg creatinine). In this study, urinary TXA_2 was not altered by exercise. Piret et al. (1990) also reported that urinary PGI_2 increased significantly with exercise. At rest PGI_2 was 55 pg/mg creatinine; PGI_2 increase to 131 pg/mg creatinine following exhaustive exercise.

In summary, a review of the research involving the effect of exercise on PGI_2 and TXA_2 indicates that circulating concentrations of both of these prostanoids may be altered by exercise. Several researchers reported that TXA_2 increased more than PGI_2, which would suggest that the PGI_2/TXA_2 ratio declines with exercise. Other studies, however, do not support this finding. There is limited evidence that the degree of change may be dependent on exercise intensity. Finally, the suggestion by some

researchers that prostanoid concentrations may be influenced by the sampling technique indicates that care must be taken when collecting samples and that interpretation of results should be made with caution.

Diet and Prostanoids

Several dietary factors are known to influence circulating prostanoids. These factors include the prostanoid substrates, oleic, linoleic and -linolenic acids, the P/S ratio and the serum lipoproteins, LDL and HDL.

Dupont (1987) reported that when dietary intake of linoleic acid is within the appropriate range PGI_2 synthesis is intact. However, when linoleic acid is deficient the oleic acid pathway becomes active. The relationship between the activity of the oleic and linoleic acid pathways is known as the triene/tetraene ratio. In mammals a triene-tetraene ratio of 0.2 in total plasma lipids is indicative of linoleic acid deficiency. Such deficiency is marked by various skin disorders in most mammals and is associated with early mortality in rats. Excessive dietary linoleic acid has also been associated with premature mortality in rats.

Dietary intake of less than three percent of total energy consumption of linoleic acid has been associated with high triene/tetraene ratios and linoleic acid deficiency. Intake of linoleic acid between three to six percent of total energy consumption has been associated with over production of prostanoids. In contrast, linoleic acid consumption between

six and ten percent of total energy consumption has been associated with normal prostanoid synthesis. Therefore, Dupont (1987) recommended that linoleic acid intake range between six and ten percent of total energy consumption.

Linoleic acid metabolism is also influenced by poly-unsaturated and saturated fat intake. In general, the lower the P/S ratio the greater the amount of linoleic acid necessary for normal prostanoid synthesis. Thus, Dupont (1987) reported that individuals with low P/S ratios should consume a greater proportion of foods rich in linoleic acid, providing the percent of total energy consumption of this nutrient does not exceed ten percent.

Lipoproteins and Prostanoids

Several studies have investigated the relationship of LDL and HDL to prostanoid synthesis. In a study by Beitz and Forster (1980), HDL was positively correlated with porcine microsome's capacity to convert PGH_2 to PGI_2. These researchers also found that LDL was inversely correlated with the conversion of PGH_2 to PGI_2. More recently, Beitz et al. (1989) studied the effects of HDL on PGI_2 and TXA_2 formation. Consistent with previous findings Beitz et al. (1989) reported that HDL stimulated the conversion of PGH_2 to PGI_2. In addition, these researchers reported that HDL inhibited the aggregating properties of TXA_2.

Fleisher et al. (1982) found that human HDL added to porcine aortic tissue cultures resulted in a significant

dose-dependent increases in PGI_2 production. No change in PGI_2 production was associated with any concentration of LDL added to cultures. An apparent relationship between the effects of LDL and HDL was found by Nordoy et al. (1978). In this study, LDL enhanced platelet aggregation while HDL inhibited the pro-aggregatory effects of LDL. No measurement of PGI_2 or TXA_2 was performed in this study.

Gryglewski and Szczeklik (1981) reported that LDL levels were associated with the accumulation of lipid peroxides. Lipid peroxide accumulation has been associated with aging and the inhibition of PGI_2 synthesis (Chang, Nagasawa, Takeguchi & Sih, 1980; Kent, Kitchell, Shand & Whorton, 1981).

In summary, there is sufficient evidence to indicate that the dietary intake of linoleic acid, saturated and polyunsaturated fat and plasma concentrations of LDL and HDL influence concentrations of prostanoids. Consequently, any investigation involving comparisons between concentrations of prostanoids should include an examination of both lipid profiles and dietary factors.

CHAPTER III

METHODS

Subjects

Twenty healthy volunteers, ten young men (25 to 35 years old) and ten older men (50 to 65 years old), from the Greensboro community participated in this study. Each prospective subject was informed of the procedures and risks associated with participation in this study, and his right to terminate participation at any time. The right to terminate participation was explained both verbally and in written form in accordance to the procedures filed with the School of Health, Physical Education, Recreation and Dance, Human Subjects review Committee (Appendices A and B).

Each prospective subject completed a medical history before he was allowed to participate (Appendix C). Subjects in the older group also obtained written permission from their family physician before they were accepted as participants (Appendix D). Individuals who smoked or had been diagnosed as having coronary heart disease, diabetes or any other chronic disease that may have influenced the results of this study were not allowed to participate. Subjects determined to have symptoms of coronary heart disease were not allowed to participate. Symptoms of coronary heart disease were determined by the guidelines in

Table 1-1 on page 2 of the Guidelines for Exercise Testing and Exercise Prescription published by the American College of Sports Medicine, 1986.

Testing Protocol

Each subject met with the researcher on three occasions. The first meeting was held for the purpose of pre-screening prospective subjects. During the second meeting each subject was required to perform a maximal exercise test; and, during the third meeting each subject participated in a sub-maximal exercise session. Subjects in the young group were tested in the Exercise Physiology Laboratory at the University of North Carolina at Greensboro. Maximal exercise testing of subjects in the older group was conducted in the Exercise Physiology Laboratory under physician supervision or in the Student Health Center at the University of North Carolina at Greensboro with a physician on the premises. Medical records for the older men tested in the Student Health Center were assessed by the health center director before these subjects were tested.

In the first meeting all subjects were informed about the procedures, risks and benefits associated with participation in the present study (Appendices A and B). This meeting took place approximately one week before the first exercise session. At this time, each subject completed a medical history (Appendix C). Each subject was asked to begin using Mazola margarine and Mazola cooking oil as a substitute for

any margarine or cooking oils they typically used. This included margarine or oils used for: baking, spreading on breads or other foods, greasing a baking or frying pan, etc. Subjects were asked to continue using Mazola margarine and cooking oil until they completed participating in the study. Subjects were asked to use these Mazola products as a means of partially controlling the dietary intake of fatty acids. The researcher emphasized that it was important for the subject not to alter other eating habits during participation in the study.

During the first meeting each older man was presented with a "Physician's Disclosure and Approval Statement" (Appendix D) which was completed and returned to the researcher before the subject was allowed to participate in the study. Older men were provided with a list of "Contraindications for Exercise Testing" (Appendix E) during the first meeting. Each older subject was instructed to deliver the list of contraindications to his physician when they met to evaluate whether or not the prospective subject was suitable for participation.

Following completion of the above procedures each subject participated in a maximal exercise test on a motorized treadmill. This test was conducted to determine maximal oxygen consumption, which was used as the basis for estimating the sub-maximal workload. Expired air was continuously collected for determination of oxygen

consumption. Samples of expired air were collected for one minute each; and, the oxygen and carbon-dioxide content of each sample was determined on a Beckmann OM-11 oxygen analyzer and a Beckmann LB-2 gas analyzer, respectively. Both analyzers were calibrated before each test with calibration gases acquired from Air Products.

During the maximal exercise test each subject began running or walking at a speed and grade estimated to be well below his maximal exercise capacity. The speed and grade were then increased every one to two minutes until the subject indicated that he could not continue or until the researcher stopped the test. Each maximal exercise test was completed within fifteen minutes following the beginning of the test. Each older man was continuously monitored by twelve-lead electrocardiography (ECG); whereas, the younger men were monitored with three-lead ECG. Electrocardiographic monitoring complied with the Guideline for Exercise Testing and Prescription published by the American College of Sports Medicine, 1986. Blood pressure was measured prior to beginning the maximal exercise test and the researcher attempted to measure blood pressure at least once during each stage of this test (Appendix F).

Following the first exercise test and before the second exercise test the subjects were asked to complete a four-day dietary food record (Bazzarre, 1983). Records were kept for two week days (i.e. tuesday and thursday) and both

weekend days. Food records were used to estimate each subject's intake of linoleic acid, saturated fat and polyunsaturated fat. The two weekdays were weighted to represent five-sevenths of the total intake during the food record period (Appendix G).

The second exercise test session was conducted within two weeks following the first test. Subjects were asked to fast overnight the evening preceding the second exercise test. Each subject rested a minimum of fifteen minutes immediately preceding the second exercise test. During the second test each subject walked or ran on the motorized treadmill for thirty minutes between 70 and 75 percent of their maximal oxygen consumption. Expired air was collected and analyzed periodically to insure that subjects were exercising at the desired intensity (Appendix H). Subjects were continuously monitored with a three-lead ECG. Each sub-maximal test was conducted between the hours of 8:00 am and 12:00 noon to control for possible diurnal variations (Nadler, J. & J. Yamamoto, 1986).

Blood Sampling

Three blood samples were drawn during the second visit using sterile Vacutainer techniques. The first blood sample (i.e. three, ten milliliter Vacutainers) was drawn at least fifteen minutes after the subject was rested, the second sample (i.e. two, ten milliliter Vacutainers) immediately following thirty minutes of exercise, and the third sample

(i.e. two, ten milliliter Vacutainers) thirty minutes following the completion of exercise. Blood was drawn from an antecubital vein with the aid of a tourniquet. Since obstruction of blood flow may alter circulating concentrations of PGI_2 and TXA_2 the tourniquet was removed before blood was collected into the last Vacutainer.

Treatment of Blood Samples

Blood samples used for the determination of prostanoids, platelet counts, hemoglobin, and hematocrit were drawn into ten milliliter Vacutainers containing .10 microliters of a fifteen percent ethylenediaminetetraacetic acid (EDTA) solution and .20 microliters of a .40 percent acetylsalicylic acid solution. Samples collected for determination of lipoproteins were collected in Vacutainers that did not contain any anticoagulants. Approximately two milliliters of whole blood was removed for the determination of platelet count, hemoglobin, and hematocrit. The remaining blood was centrifuged immediately at 3000 rpm for fifteen minutes. Plasma was extracted and frozen at -70 C until samples were assayed.

Analysis of Blood Samples

Platelet counts were determined manually using a Unopette Microcollection System and hemocytometry. Hemoconcentration was determined by the procedures outlined by Dill and Costill (1974). Hemoglobin was evaluated according to the procedures outlined by Sigma Diagnostics Co. Inc.

Plasma levels of PGI_2 and TXA_2 (i.e. 6-keto-$PGF_{1\alpha}$ and TXB_2, the stable metabolites of PGI_2 and TXA_2, respectively) were determined by ^{125}I radioimmunoassay. Extraction of the prostanoids from plasma was conducted in the following manner. Seventy-five microliters of a seven percent solution of hydrogen chloride was added to one-half milliliter of plasma in order to lower the pH of the plasma to between three and four. Three volumes of ethyl acetate were added to the plasma; and, each sample was mixed thoroughly on a vortex mixer. The aqueous and organic phases were separated by centrifugation for fifteen minutes at 3000 rpm. The upper organic phase containing the prostanoids was removed and the ethyl acetate was allowed to evaporate.

Assays were conducted according to the procedures outlined by Advanced Magnetics, Inc. All frozen reagents were thawed for 30 minutes before they were used. Lyophilized reagents were reconstituted with phosphate buffer provided by Advanced Magnetics, Inc. Round bottom polypropylene tubes measuring 12 X 75 mm were used for all samples and standards. Serial dilutions of the stock prostanoid concentrate (100 ng/ml) were distributed into a series of six concentrations (i.e. 4.1, 12.3, 37.0, 111.0, 333.0, and 1000.0 pg/0.1ml) to create an assay standard. Tubes were labeled in duplicate for determination of total count, non-specific binding (NSB), blank (Bo), standards and

samples. The remaining assay procedures were conducted as follows:

1. 200 ul of buffer was added to NSB and sample tubes.
2. 100 ul of buffer was added to Bo tubes.
3. 100 ul of standard was added to the twelve tubes labeled for assay of the standard.
4. 100 ul of sample was added to the remaining tubes.
5. 100 ul of the tracer was added to all tubes.
6. 100 ul of antiserum was added to each tube.
7. Each tube was vortexed and refrigerated for twenty to twenty-four hours.
8. 500 ul of magnetic goat anti-rabbit was added to each tube, except for the two total count tubes.
9. Tubes were incubated for fifteen to twenty minutes at room temperature.
10. With the exception of the total count tubes, each tube was centrifuged at 3000 rpm at 4 C for twenty minutes.
11. Supernatant was decanted from each tube, except for total count tubes, and discarded as radioactive waste.
12. Tubes were gently blotted on a plastic lined paper towel to remove remaining supernatant.
13. Concentration of radioactivity present in each tube was counted in a gamma counter.

Thromboxane A_2 concentrations were adjusted for exercise-induced alterations in platelet count. Both PGI_2 and TXA_2 were adjusted for alterations in hemoconcentration. The PGI_2/TXA_2 ratio was computed by dividing the plasma concentration of PGI_2 by the adjusted plasma concentration of TXA_2.

Lipid Profile Assessment

Serum total cholesterol, HDL, and triglycerides were determined by enzymatic procedures employed at Moses Cone Memorial Hospital in Greensboro, North Carolina. Plasma LDL was estimated by Moses Cone Memorial Hospital from the total cholesterol and triglyceride values.

Nutritional Assessment

Food records were analyzed first with Nutritionist III (N-Squared Computing, 1985) to estimate the average daily intake of linoleic acid, saturated fat and polyunsaturated fat. When Nutritionist III provided insufficient fat composition data, Food Values of Portions Commonly Used (Jean A. T. Pennington, 1989), was used to further estimate linoleic acid, saturated fat and polyunsaturated fat intake.

Statistical Analysis

In order to determine if there were statistically significant differences between VO_2max, sub-maximal exercise VO_2's and exercise intensity when expressed as a percentage of VO_2max the values from the two different age groups were compared using t-test.

The effects of age and exercise PGI_2/TXA_2 ratios, plasma PGI_2, and plasma TXA_2 in the two different age groups at rest, during exercise and during recovery were assessed with a 2 X 3 repeated measures analysis of variance. The Geisser-Greenhouse correction factor was used to adjust the degrees of freedom for the F-test (Glass & Hopkins, 1984). When significant main effects were found, separate pairwise comparisons were made using Tukey's multiple comparison technique.

The effect of dietary linoleic acid, P/S ratio, and serum HDL and LDL concentrations on the PGI_2/TXA_2 ratios, plasma PGI_2, and plasma TXA_2 were examined as follows: a separate 2 x 3 repeated measures analysis of covariance was conducted using each of the above variables (i.e. dietary linoleic acid, P/S ratio, plasma HDL and plasma LDL) as covariates.

Subject's with PGI_2/TXA_2 ratios more than two standard deviations beyond the group mean were removed from the analysis. Statistical significance was set at the 0.05 probability level.

CHAPTER IV

RESULTS

Treatment of Individual Data

Twenty subjects, ten young men (25 to 30 years old) and ten older men (50 to 62 years old), participated in the present study. Data from two subjects, one young man and one older man, were not included in the analysis. These data were removed because one PGI_2/TXA_2 ratio for each subject was more than two standard deviations beyond group means; and, these ratios were regarded physiologically implausible when compared to other ratios.

Exercise Characteristics

Assessment of the exercise data by the t-test procedure indicated that nine young men (27.8 $\pm$ 0.8 years old) and nine older men (55.4 $\pm$ 1.3), differed on VO_2max ($p < 0.01$) and highest heart rate achieved during the maximal exercise test ($p < 0.001$). Mean maximal heart rates were 101.4% and 104.0% of the age predicted maximal heart rates for the young and older groups, respectively. The VO_2's for the older men during the sub-maximal exercise session were lower than the younger men at approximately five ($p < 0.001$) and twenty minutes ($p < 0.05$) of exercise. However, VO_2's during sub-maximal exercise, when expressed as a percentage of VO_2max, did not differ significantly between the two groups (Table

4). Body weights between the two groups were significantly different ($p < 0.05$). The mean body weight for the young men was 153.2 ± 6.4 lb (69.6 ± 2.8 kg) and 180.2 ± 7.1 lb (81.9 ± 3.2 kg) for the older men.

Table 4.

Exercise Characteristics

Group	HRmax[a] (bpm)	VO_2max (ml/kg/min)	VO_2 5 min	VO_2 20 min	%VO_2max 5 min	%VO_2max 20 min
Young Adult	194.9** ± 2.4	53.4* ± 2.8	36.9* ± 1.4	39.1** ± 1.7	69.6 ± 1.8	73.4 ± 0.8
Older Adult	171.2** ± 2.9	40.1* ± 2.5	28.2* ± 2.1	28.5** ± 1.5	69.7 ± 2.1	74.0 ± 1.2

Between group differences: *(p < .01); **(p < .001).

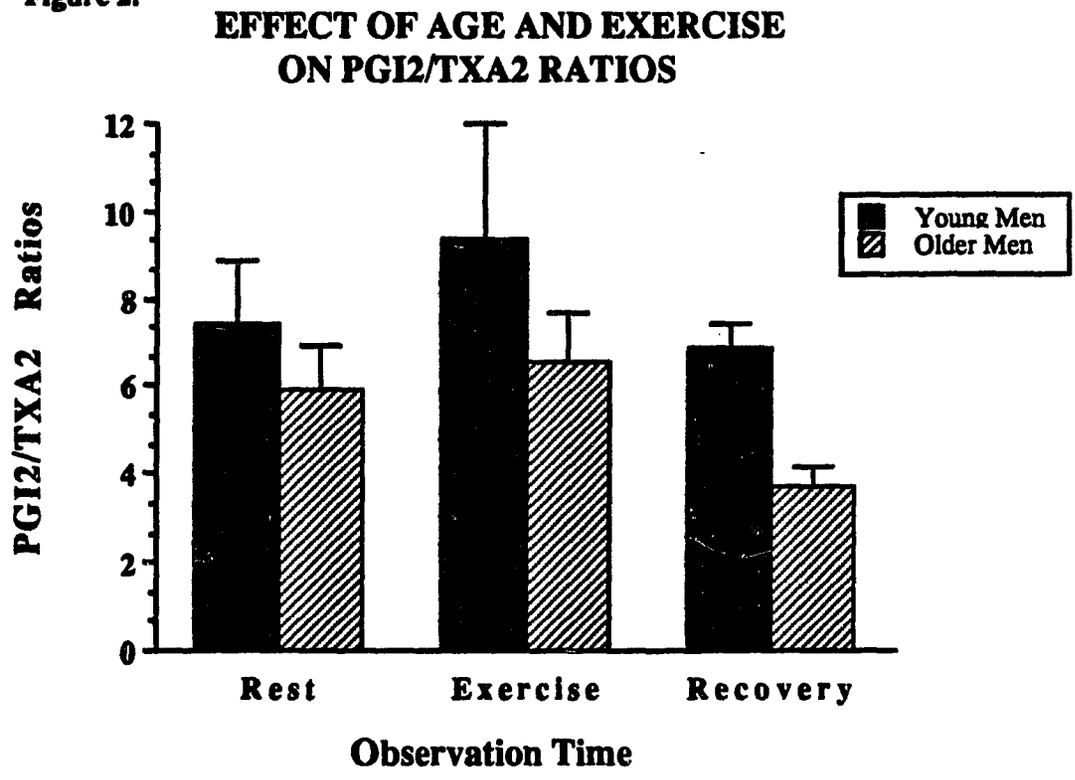
[a]Highest heart rate achieved during maximal exercise test.

PGI_2/TXA_2 Ratios

Analysis of variance indicated that age had a significant ain effect on PGI_2/TXA_2 ratios ($F = 5.92$; $p = 0.027$). No significant differences in PGI_2/TXA_2 due to the main effect of exercise were found.

Resting PGI_2/TXA_2 ratios were 7.44 ± 1.42 for the young men and 5.90 ± 0.99 for the older men (Figure 2). The mean

Figure 2.



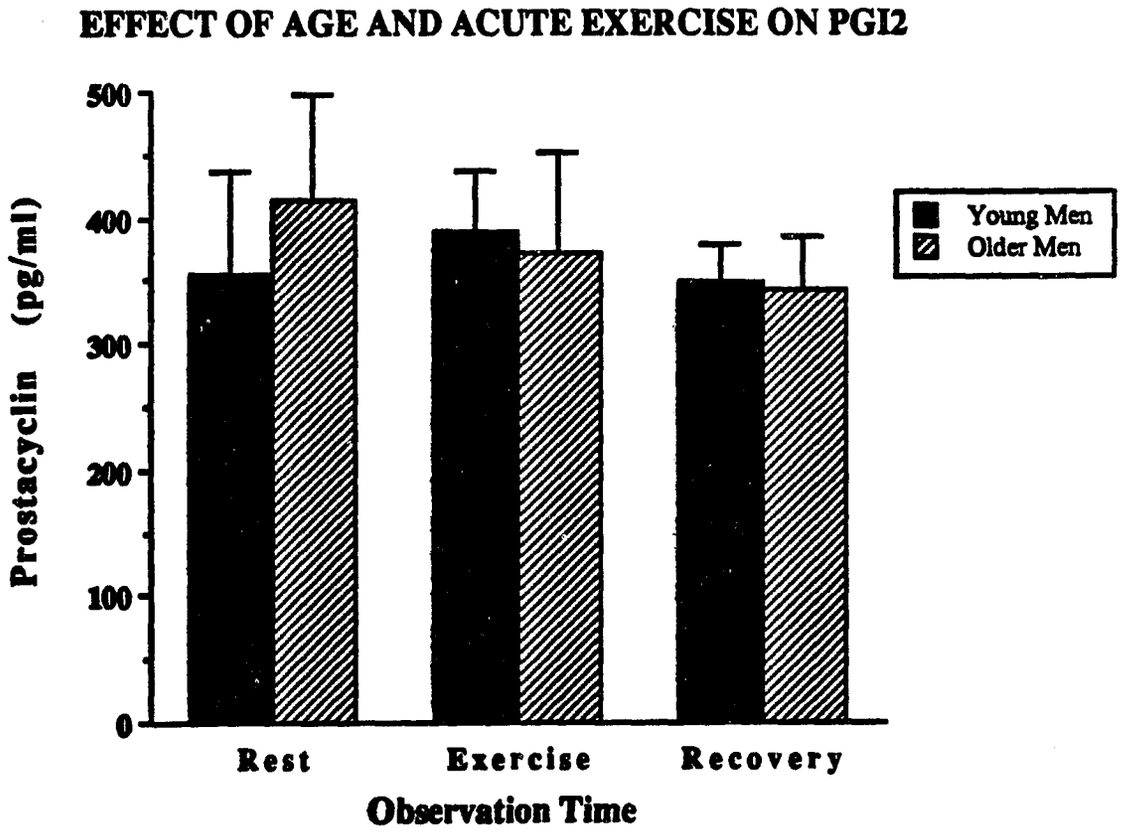
resting ratio for the young group was 26.0% higher than the resting ratio for the older group; the difference was not significant. The mean ratio for the young men immediately following exercise increased 25.4% to 9.33 $\pm$ 2.69; and, the mean ratio for the older men increased 10.3% to 6.51 $\pm$ 1.14. These increases were not significant when compared to resting values. The exercise ratio for the young group was 43.3% greater than the exercise ratio for the older group; but, the difference was not significant. Thirty minutes following exercise the older men's ratio was 3.69 $\pm$ .47, which was 37.5% below their resting ratio. The young men's recovery

ratio was 6.83 ± .57, or 8.2% below their resting ratio. The exercise values did not differ significantly from rest. The young group's recovery ratio was 85.2% greater than the older group's, although the difference statistically significant.

PGI_2 Concentrations

Analysis of variance indicated that neither age ($F = 0.03$; $p = 0.857$) nor exercise ($F = 0.43$; $p = 0.654$) had any main effect on plasma PGI_2 (Figure 3). Resting concentrations of PGI_2 were 355.56 ± 82.38 pg/ml and 412.89 ± 85.40 pg/ml for the young and older men, respectively.

Figure 3.



Immediately after exercise PGI_2 concentrations were 389.05 ± 47.01 pg/ml for the young group and 372.44 ± 79.80 pg/ml for the older group. Thirty minutes following exercise PGI_2 concentrations were 349.57 ± 29.48 pg/ml and 342.85 ± 42.03 pg/ml for the young and older men, respectively. No significant age group or exercise-induced differences were found for PGI_2 concentrations.

TXA_2 Concentrations

Analysis of variance indicated that age had a significant main effect on plasma TXA_2 ($F = 4.52$; $p = 0.049$). No significant differences in concentrations of TXA_2 due to the main effect of exercise were found ($F = 0.58$; $p = 0.565$).

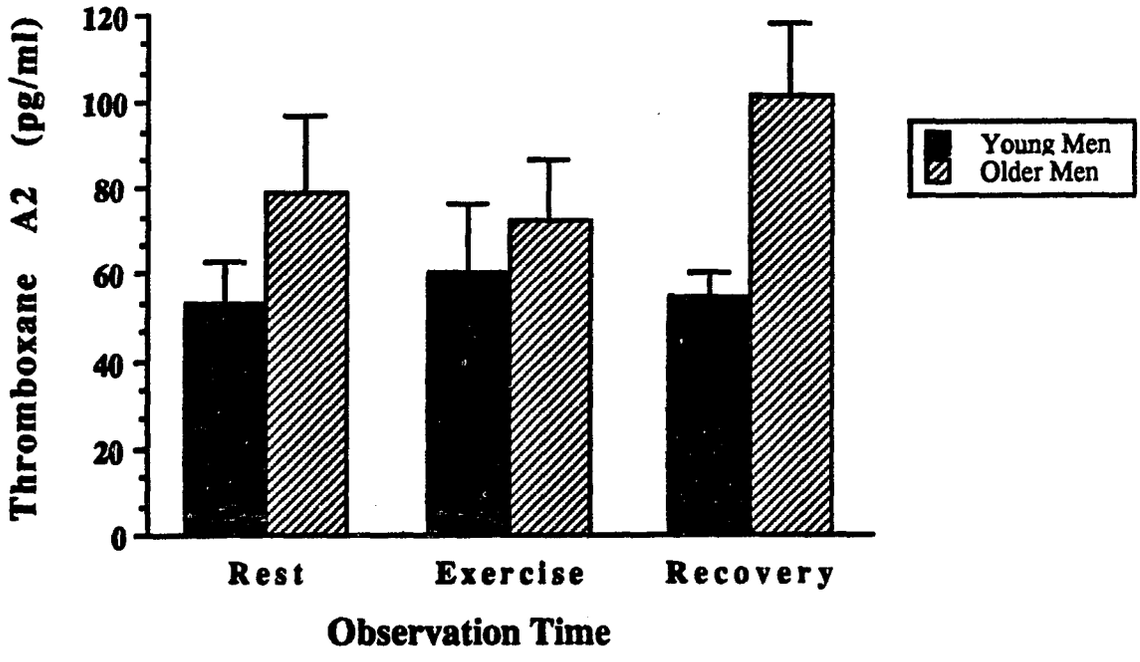
Resting TXA_2 concentrations were 53.33 ± 9.62 pg/ml and 79.00 ± 18.15 pg/ml for the young and older groups, respectively (Figure 4). These values were not significantly different, although the TXA_2 concentrations found in the older men was approximately forty-eight percent higher than the young men's values. Immediately following thirty minutes of exercise circulating concentrations of TXA_2 were 60.29 ± 15.94 pg/ml for the young group and 72.79 ± 13.71 pg/ml for the older group. These values were not significantly different from each other, nor were they significantly different from resting values.

Thirty minutes following exercise the TXA_2 concentration for the young men was 54.44 ± 6.19 pg/ml. The TXA_2 concentration for the older men was 101.71 ± 16.38 pg/ml, or

nearly two-fold greater than the value for the young men, although these values were not significantly different. No significant differences in TXA_2 concentrations due to the effect of exercise were found within either age group. Exercise appears to have a dramatic influence on TXA_2 in older men when concentrations are measured thirty minutes following exercise.

Figure 4.

EFFECT OF AGE AND ACUTE EXERCISE ON TXA2



Lipid Profiles

Group values for total cholesterol were significantly different ($p < 0.05$) when analyzed by t-test procedures. The older men had higher total cholesterol values when compared

to the younger men. Values for HDL, LDL and triglycerides were not different at the 0.05 alpha level (Table 5). The older group's LDL values were eighteen percent higher than the younger group, although the difference was not significant.

Table 5.

Lipid Profiles

Group	Total Cholesterol (mg/dl)	HDL (mg/dl)	LDL (mg/dl)	Triglycerides (mg/dl)
Young Adult	176.8* ± 8.7	52.2 ± 5.6	108.3 ± 5.9	81.8 ± 14.7
Older Adult	218.1* ±12.6	52.6 ± 4.0	132.0 ±12.6	216.2 ± 209.2

Between group differences: *($p < .05$).

HDL, LDL, and Total Cholesterol as Covariates

High density lipoprotein was not used as a covariate since there was not a significant difference between group values. The age group-related main effect differences between PGI_2/TXA_2 ratios for each group were eliminated when LDL was used as a covariate. The p-value for the effect of the between subject factor, group, on PGI_2/TXA_2 ratios was

0.101 (F = 3.08); and, the p-value for the regression factor, LDL, was 0.115 (F = 2.83). The age group-related main effect differences between TXA_2 concentrations for each group were eliminated when LDL was used as a covariate. The p-value for the effect of group on TXA_2 concentrations was 0.162 (F = 2.18); and the p-value for LDL was 0.640 (F =0.23).

Total cholesterol was used as a covariate, since a significant difference between the young and older men's values was obtained. The age group-related main effect differences between both PGI_2/TXA_2 ratios and TXA_2 concentrations were eliminated when total cholesterol was used as a covariate. The p-value for the effect of the between subject factor, group, on PGI_2/TXA_2 ratios was 0.117 (F = 2.00); whereas, the p-value for the regression factor, LDL, was 0.290 (F = 1.20). The p-value for the effect of group on TXA_2 concentrations was 0.144 (F = 2.37); and, the p-value for LDL was 0.764 (F = 0.09).

Dietary Characteristics

Group values for mean daily intake of K-calories and grams of fat were not significantly different. Mean daily intake of linoleic acid, when expressed as a percentage of mean daily total caloric intake was not significantly different between the two groups.

Group values for P/S ratios approached a statistically significant difference (p = 0.09). This tendency was characterized by a significant difference in the mean daily

intake of polyunsaturated fat in grams ($p < 0.05$). Daily intake of saturated fat was not different (Table 6).

Table 6.

Dietary Characteristics

Group	Mean Daily Kcal[a]	Fat[b] (g)	Poly[c] Fat (g)	Sat[d] Fat (g)	P/S Ratio	Linoleic[e] Acid (% Kcal)
Young Adult	2611.6 ± 251.2	86.7 ± 10.1	38.42* ± 4.62	28.66 ± 5.26	1.66 ± .36	5.74 ± .07
Older Adult	2404.0 ± 214.5	83.7 ± 10.2	25.95* ± 3.11	26.33 ± 2.53	1.00 ± .11	4.98 ± .02

Between group differences; *($p < 0.05$).

[a]Mean daily intake of Calories.

[b]Mean daily intake of fat (grams)

[c]Mean daily intake of polyunsaturated fat (grams).

[d]Mean daily intake of saturated fat (grams).

[e]Mean daily intake of linoleic acid (grams), expressed as a percentage of mean daily intake of Calories.

Linoleic Acid, P/S Ratios, and Polyunsaturated Fat as Covariates

Linoleic acid was not used as a covariate since there was not a significant difference between group values. The age

group-related main effect difference between PGI_2/TXA_2 ratios for each group was still present when P/S ratios were used as covariates. The p-value for the effect of the between subject factor, group, on PGI_2/TXA_2 ratios was 0.050 (F = 4.54); and, the p-value for the regression factor, P/S fat ratios, was 0.963 (F = 0.00). The age group-related main effect differences between TXA_2 concentrations for each group were eliminated when P/S ratios were used as covariates. The p-value for the effect of group on TXA_2 concentrations was 0.087 (F = 3.36); and the p-value for P/S ratios was 0.913 (F = 0.01).

Mean daily intake of polyunsaturated fat in grams was used as a covariate, since a significant difference between the young and older group's values was found. The age group-related main effect difference between PGI_2/TXA_2 ratios for each group remained when polyunsaturated fat was used as a covariate. The addition of the polyunsaturated fat did not make a significant contribution to the analysis as the error term actually increased from 14.23 to 14.71. The p-value for the effect of the between subject factor, group, on PGI_2/TXA_2 ratios was 0.030 (F = 5.75); and, the p-value for the regression factor, polyunsaturated fat, was 0.497 (F = 0.48). The age group-related main effect difference between TXA_2 concentrations for each group was eliminated when polyunsaturated fat was used as a covariate. The p-value for the effect of group on TXA_2 concentrations was 0.070 (F =

3.80); and the p-value for polyunsaturated fat was 0.716 (F = 0.14).

Summary of the Results

The heart rate data suggest that both groups achieved maximal exercise capacities during the maximal exercise test. The relative intensities maintained during the sub-maximal exercise test were the same for both groups, although the absolute intensities were significantly different.

Resting and immediate post-exercise PGI_2/TXA_2 ratios were not different between the two groups. There was approximately a two-fold difference between the young and older group's PGI_2/TXA_2 ratios thirty minutes following exercise, although the difference was not statistically significant. The apparent difference was characterized by a 28.7% increase in TXA_2 above resting concentrations in the older group as compared to a essentially no change in the young group thirty minutes after exercise.

Total cholesterol and dietary intake of polyunsaturated fat were significantly different between the two groups. Linoleic acid intake, expressed as a percentage of total caloric intake, and serum LDL between the two groups approached statistically significant differences. None of the lipoprotein or dietary characteristics had a significant impact on the statistical analysis when they were used as covariates.

CHAPTER V

DISCUSSION

Clinical manifestations of coronary heart disease may be initiated by platelet aggregation resulting from platelet contact with damaged endothelial tissue (Davies, & Thomas, 1984; Freeman, Williams, Chisholm, & Armstrong, 1989). Monocada and Vane (1979), suggested that the ratio between circulating concentrations of PGI_2 and TXA_2 has an important role in determining the extent of platelet aggregation; and, the lower the PGI_2/TXA_2 ratio the greater the predisposition towards platelet aggregation. Age, exercise, and diet may have an indirect role in platelet aggregation as consequence of their influence on PGI_2/TXA_2 ratios. This study was intended to elucidate the combined effects of age and exercise on PGI_2/TXA_2 ratios. Dietary and lipid profile differences existing between the two groups were controlled for statistically.

Exercise Characteristics

The statistically significant difference between maximal heart rates reported for the young and older men resulted in rejection of the hypothesis that there would be no difference between the groups. Mean maximal heart rates were 101.4% and 104.0% of the age predicted maximal heart rate for the young and older men, respectively. The highest heart rates

achieved during the maximal exercise test were within four percent of age predicted values for each group, suggesting that the subjects achieved maximal exercise capacities during this test.

Mean VO_2max's for the two groups were significantly different. The hypothesis that the two groups would not have different VO_2max's was rejected. There was not a significant difference between the groups when sub-maximal exercise intensity was expressed as a percentage of the maximal exercise intensity. The hypothesis that the two age groups would not have different sub-maximal exercise intensitites was accepted.

Mean VO_2max for the young men was 53.42 $\pm$ 2.8 ml/kg/min, indicating that this group of subjects was in the "excellent" oxygen consumption category (Pollock, & Blair, 1981). Individual VO_2max's for the young men ranged from "fair" (41.14 ml/kg/min) to "excellent" (66.80 ml/kg/min). Mean VO_2max for the older men was 40.14 $\pm$ 2.5 ml/kg/min, placing this group in the "very good" oxygen consumption category. Individual VO_2max's for the older men ranged from "fair" (28.50 ml/kg/min) to "excellent" (52.30 ml/kg/min). The range of VO_2max data (i.e. 26.66 and 23.80 ml/kg/min in the young and older men, respectively) within groups indicates that each group was somewhat heterogenous. The heterogeneity of the VO_2max data combined with the fact that significant correlations were found between VO_2max and

recovery PGI_2/TXA_2 ratios ($r = .67$; $p = 0.001$), VO_2max and resting TXA_2 concentrations ($r = -.44$; $p = 0.036$), and VO_2max and recovery TXA_2 concentrations ($r = -.67$; $p = 0.001$) suggest that aerobic fitness may influence PGI_2/TXA_2 ratios via plasma TXA_2 concentrations. The VO_2max data was added as a covariate in the analysis of the effect of age and exercise on PGI_2/TXA_2 ratios and TXA_2 concentrations since significant correlations were found to exist between these variables. Addition of the VO_2max data did not have a significant influence on the results. Several factors including the small sample size and a lack of accountability and control over the exercise training habits of subjects may have led to the low correlations found between the two groups. Exercise training status may correlate better with prostanoid concentrations, since VO_2max is not always a satisfactory indicator of exercise training.

Rauramaa et al. (1984) reported that improvements in VO_2max following an eight week exercise training program were associated with increased PGI_2 and decreased TXA_2 concentrations at rest. These researchers also reported that the increase in PGI_2 was positively related to alterations in the HDL_2 subfraction. Although Rauramaa et al. (1984) reported that LDL decreased significantly, they did not state if this reduction was significantly correlated with the decline in TXA_2.

In contrast to the findings reported by Rauramaa et al. (1984), Symons (1984) found that increased VO_2max following exercise training was not associated with any change in resting prostanoid concentrations. Symons (1984) did not find a significant change in HDL following exercise training. In the present study, HDL concentrations were not significantly different between groups; and, HDL_2 subfractions were not measured.

Neither Symons (1984) nor Rauramaa et al. (1984) investigated the effect of exercise training on prostanoid concentrations during or following an acute bout of exercise. Although data from the present investigation suggest that a significant negative correlation exist between aerobic capacity and TXA_2 concentrations further research is needed to determine if this is just an association or a true cause and effect relationship. Additional research investigating the effects of exercise training on prostanoid concentrations appears to be warranted.

Effect of Age and Exercise on PGI_2/TXA_2 Ratios

Analysis of variance indicated that age had a significant main effect on PGI_2/TXA_2 ratios ($p < 0.05$). Thus, the hypothesis that age had no effect on PGI_2/TXA_2 ratios was rejected. No significant differences in PGI_2/TXA_2 due to the main effect of exercise were found; and, the hypothesis that exercise had no effect on PGI_2/TXA_2 ratios remained tenable.

Resting PGI_2/TXA_2 Ratios

The presentation of PGI_2/TXA_2 ratio data by other researchers are limited. Mean data can be used to compute PGI_2/TXA_2 ratios, although such computations may provide erroneous data. For example, when the mean resting data for the young men in the present study were used to compute the PGI_2/TXA_2 ratio the value was 6.68. When individual data were used to compute ratios and the mean was calculated from these individual ratios the PGI_2/TXA_2 ratio was 7.44. For the purposes of this discussion PGI_2/TXA_2 ratios pertaining to other research will be calculated using mean data, unless the researchers provided individual data.

Resting PGI_2/TXA_2 ratios were not significantly different between the two groups in the present study. Thus, the hypothesis that there would not be a significant difference between the two group's resting ratios was accepted. The mean PGI_2/TXA_2 ratio for the young men (7.44 ± 1.42) was twenty-six percent higher than the mean ratio for the older men (5.90 ± 0.99). A similar difference between two age groups was computed from the mean prostanoid data reported by Vericel et al. (1988). The PGI_2/TXA_2 ratio for their young group was 4.62; whereas, the ratio for their older group was 3.75. The PGI_2/TXA_2 ratio for their young group was twenty-three percent greater than the ratio for their older group.

The derived PGI_2/TXA_2 ratios in the study by Vericel et al. (1988) were characterized by elevated concentrations of both prostanoids in the older group. Only the PGI_2 concentrations, which were 63% higher in their older group, were significantly different between the two groups, although the TXA_2 concentrations were two-fold greater in their older group. Data from the present study were similar, with plasma PGI_2 16.2% higher and TXA_2 concentrations 48.1% higher in the older group.

Reilly and FitzGerald (1986) reported that both urinary concentrations of PGI_2 and TXA_2 were higher in older adults when compared to young adults. These findings are in agreement with data from the present study and with the results reported by Vericel et al. (1988); however, when the mean values presented by Reilly and FitzGerald are used to compute PGI_2/TXA_2 ratios the older group appears to have the more favorable ratio. The PGI_2/TXA_2 ratios were .796 and .883 for the young and older adults, respectively.

The report by Vericel et al. (1988) and the present study suggest that age-related alterations in PGI_2/TXA_2 ratios may predispose older adults to platelet aggregation under resting conditions; whereas, Reilly and FitzGerald (1986) reported that PGI_2/TXA_2 ratios were only slightly different between the two age groups. Age-related alterations were characterized by increases in both PGI_2 and TXA_2 concentrations in each of the studies reviewed. Thus, one

might conclude that the increased risk of platelet aggregation associated with an age-related increase in TXA_2 is attenuated by a concomitant increase in PGI_2.

Exercise and Recovery PGI_2/TXA_2 Ratios

There was not a significant difference between the two group's PGI_2/TXA_2 ratios immediately following exercise, although the ratio for the young men was 43.3% greater than the ratio for the older men. The hypothesis that there would not be a significant difference between the young and older men's ratios immediately following exercise was accepted. The large degree of variability among the young men's PGI_2/TXA_2 ratios immediately after exercise and the small sample size may have contributed to the lack of significance.

Following exercise the PGI_2/TXA_2 ratios for each group tended to rise when compared to the resting values. The increases above resting values were approximately twenty-five percent for the young men and ten percent for the older men. These data suggest that, when compared to resting values, neither group was more predisposed to platelet aggregation immediately after exercise.

Metha et al. (1983) found a greater than two-fold increase in PGI_2/TXA_2 ratios immediately following exercise. These exercise-induced alterations were characterized by increases in both plasma PGI_2 and TXA_2 concentrations. In the present study, PGI_2/TXA_2 ratios in the young group tended to increase immediately after exercise; and, the increase was

marked by a slight increase in both plasma PGI_2 and TXA_2. The results reported by Metha et al. (1983) are consistent with the data from the young group involved in the present study.

Taniguchi et al. (1984) reported contrasting results. These researchers found that PGI_2/TXA_2 ratios declined by approximately 40% immediately following twelve minutes of treadmill exercise (Taniguchi, Furui, Yamauchi, & Sotobata, 1984). The exercise-induced changes reported by Taniguchi et al. (1984) were characterized by an 11% decline in PGI_2 and a 48% increase in TXA_2. In contrast, during the last minute of exercise both PGI_2 and TXA_2 concentrations were within one percent of resting values (Taniguchi, Furui, Yamauchi, & Sotobata, 1984). The inconsistencies among the data presented by Taniguchi et al. (1984) may indicate that a large degree of experimental error was present in their study.

In the present study, the PGI_2/TXA_2 ratio for the young men thirty minutes following exercise was similar to their resting ratio. Both PGI_2 and TXA_2 returned to near resting levels for the young group at thirty minutes recovery. In the older men, the PGI_2/TXA_2 ratio dropped 37.5% below the resting value. This change was characterized by a 28.7% increase in TXA_2 concentrations above resting concentrations. The ratio in the younger group was approximately two-fold greater than the ratio for the older group; however, the

difference between the two group's ratios were not statistically significant. Thus, the hypothesis that there was not a significant difference between recovery ratios for the young and older men was accepted. The large degree of variability associated with the young men's immediate post-exercise data may have contributed to the fact that a significant between group difference was not found thirty minutes following exercise.

These data suggest that thirty minutes after exercise, older men may be more predisposed to platelet aggregation than young men. The fact that the older group's PGI_2/TXA_2 ratios thirty minutes post-exercise were lower than their resting ratios provides additional evidence that some older men may be more susceptible to platelet aggregation after exercise.

Discussion of the potential underlying mechanisms associated with the apparent age related differences in PGI_2/TXA_2 ratios is presented later in this chapter. However, one observation made while comparing the results of the present study with another investigation will be presented here.

Metha et al. (1983) investigated whether or not PGI_2 and TXA_2 concentrations were different at rest and following exercise in persons with known coronary heart disease as compared to apparently healthy individuals. The PGI_2/TXA_2 ratios computed from mean data indicated that fifteen to

thirty minutes following exercise the apparently healthy subject's ratios returned to near resting levels; whereas, the ratios for the subjects with coronary heart disease fell below resting levels (Metha, Metha, & Horalek, 1983). The exercise-induced alterations in PGI_2/TXA_2 ratios for some of the older subjects in the present study may be similar to the responses exhibited by persons with coronary artery disease, suggesting that some older persons in the present study may have coronary artery disease or manifest some of the mechanisms leading to coronary artery disease. This possibility cannot be completely ruled out, although careful subject screening was employed to eliminate potential subjects with this disorder.

Effect of Age and Exercise on PGI_2

Analysis of variance indicated that neither age nor exercise had any main effect on plasma PGI_2. These results supported the null hypothesis.

Resting PGI_2 Concentrations

In the present study resting concentrations of PGI_2 between the two age groups were not significantly different. This finding is consistent with results reported by Hanley and May (1985); but, it does not concur with reports by Reilly and Fitzgerald (1986) or Vericel et al. (1988). Hanley and May (1986) reported that there was no significant relationship between age and PGI_2 synthesis in saphenous venous tissue removed from men and women treated for varicose

veins. The researchers analyzed the data for men and women separately. There is a lack of evidence indicating whether or not vascular changes associated with varicose veins alters prostanoid synthesis; and, data collected from smokers was included in the analysis. Several researchers have reported that nicotine and smoking inhibits the synthesis of PGI_2 (Pittilo, Mackie, Rowles, Machin, & Woolf, 1982; Sonnenfeld, & Wennmalm, 1980; Wennmalm, 1980).

Reilly and FitzGerald (1986) reported that resting urinary PGI_2 concentrations were higher among adults 50 to 88 years old when compared to adults 21 to 39 years old. Reilly and FitzGerald (1986) did not measure circulating concentrations of PGI_2; but, under resting conditions, urinary concentrations of PGI_2 paralleled circulating PGI_2 concentrations (Vericel et al., 1988).

Vericel et al. (1988) reported that both urinary and serum PGI_2 concentrations at rest were higher in 78 to 94 year old adults when compared to 25 to 35 year old adults. Serum concentrations of PGI_2 were approximately two-fold higher in the older adults. In the present study, resting plasma PGI_2 concentrations in the older men were approximately sixteen percent greater than PGI_2 concentrations found in the young men.

One factor that may have contributed to the contrasting results between the present study and other studies is that the age range between the young and old groups was greater

in the other studies. The ranges were approximately 56 years in the study by Vericel et al. (1988), 39 years in the study by Reilly and FitzGerald (1986), and 28 years in the present study. Vericel et al. (1988) reported finding a significant difference between the two age groups. Reilly and Fitzgerald (1986) did not find a significant difference; but, urinary PGI_2 concentrations in the older group were 62% greater than in the younger group. These reports suggest that age-related increases in PGI_2 may not obtain statistically significant levels until individuals reach 65 to 70 years old.

Another factor that might have contributed to the differences between the present study and the study by Vericel et al. (1988) is that group activity and fitness levels may have been different between these studies. Vericel et al. (1988) did not provide any information regarding the fitness or activity levels of their subjects. In the present study there were similar numbers of training and non-training individuals in each group; however, subjects represented a wide range of aerobic fitness categories. Future research should attempt to account for the effect exercise training may have on prostanoid concentrations since Rauramaa et al. (1984) reported that exercise training may lead to significant alterations in PGI_2.

Both the lack of an age-related difference in resting PGI_2 concentrations found in the present study and the

existence of age-related increases reported by other researchers (Vericel et al., 1988) seem to contradict data gathered from studies conducted in vitro. Researchers have consistently reported that fresh aortic tissue samples from older animals (i.e. rats and swine) release significantly less PGI_2 than samples from younger, mature animals (Chang, Murota, & Nakao, 1980; Chang, & Tai, 1983; Giani, Masi, & Galli, 1985; Kent, Kitchell, Shand, & Whorton, 1981; Menconi, Taylor, Martin, & Polgar, 1987). The lack of agreement between these two bodies of literature may be related to the fact that PGI_2 synthesis is influenced by many substances found in the circulation. Some of these substances include: arachidonic acid, adenosine diphosphate, collagen, bradykinin (Menconi, Taylor, Martin & Polgar, 1987), HDL, LDL (Beitz, & Forster, 1980), and the essential fatty acids (DuPont, 1987). Beitz and Forster (198) have shown that PGI_2 synthesis from PGH_2, a component of the arachidonic acid cascade, is enhanced in the presence of HDL and inhibited by LDL. Research conducted in vitro may limit the tissue exposure to one or more of the above substances. Further investigation of PGI_2 synthesis from animal tissues bathed in different mediums is needed. Tissue samples taken from animals following acute or chronic exercise may also provide valuable information regarding the effects of exercise on PGI_2 synthesis.

Exercise and Recovery PGI_2 Concentrations

No significant changes in plasma PGI_2 concentrations resulting from exercise were found in the present study. These results concur with findings reported by several other researchers (Carter, Ready, Singhroy, Duta, & Gerrard, 1989; Taniguchi, Furui, Yamauchi, & Sotobata, 1984; Todd, Goldfarb, & Boyer, 1990). Other researchers have reported that PGI_2 concentrations increase as a result of exercise (Demers, Harrison, Halbert, & Santen, 1981; Metha, Metha, & Horalek, 1983; Piret et al., 1990; Viinikka, Vuori, & Ylikorkala, 1984).

Closer investigation of the exercise protocols utilized in other studies suggest that alterations in PGI_2 concentrations may be influenced by exercise intensity and duration. Studies associated with no significant changes in PGI_2 concentrations involved sub-maximal exercise (Carter, Ready, Singhroy, Duta, & Gerrard, 1989; Taniguchi, Furui, Yamauchi, & Sotobata, 1984; Todd, Goldfarb, & Boyer, 1990). Significant alterations in PGI_2 concentrations occurred when subjects exercised at maximal intensity (Metha, Metha, & Horalek, 1983; Piret et al., 1990). Subjects in the study by Viinikka et al. (1984), exercised to exhaustion; but, the only significant alteration in PGI_2 was found after seven minutes of exercise. Immediately following exercise PGI_2 concentrations returned to near resting concentrations.

The impact of long duration exercise on PGI_2 concentrations remains unclear. Demers et al. (1981), reported that plasma PGI_2 concentrations were significantly elevated following a marathon run; whereas, Carter et al. (1989) found no change after a 21 or 42 kilometer run. One difference in these two studies was that Demers et al. (1981) did not expose fresh whole blood samples to a prostanoid inhibitor.

Sub-maximal exercise, thirty minutes or less in duration, has not been associated with significant alterations in PGI_2 concentrations (Carter, Ready, Singhroy, Duta, & Gerrard, 1989; Taniguchi, Furui, Yamauchi, & Sotobata, 1984; Todd, Goldfarb, & Boyer, 1990). The results from this study are consistent with the results from previous investigations.

No significant age-related differences in PGI_2 concentrations were found immediately following exercise or thirty minutes after exercise. Other research involving the effects of age on PGI_2 during or following exercise were not found. Among the studies investigating the effects of exercise on PGI_2 concentrations mean age groups ranged from 22.2 ± 0.06 years old (Piret et al., 1990) to 50 ± 4.0 years old (Metha, Metha, & Horalek, 1983). These researchers reported similar results, with PGI_2 concentrations significantly increasing following exercise (Piret et al., 1990; Metha, Metha, & Horalek, 1983). The results of the present study suggest that age has no influence on plasma

PGI_2 concentrations immediately following thirty minutes of exercise at 70% to 75% of VO_2max or thirty minutes after exercise. The difference between the mean age for the two groups in this study was 27.6 years. Additional research investigating the effects of exercise on PGI_2 concentrations between groups representing a wider age range is warranted, especially since other researchers have reported that resting PGI_2 concentrations may differ between subjects from different age groups (Vericel, Croset, Sedivy, Courpron, Dechavanne, & LeGarde, 1988).

Effect of Age and Exercise on TXA_2

Analysis of variance indicated that age had a significant main effect on plasma TXA_2 ($p < 0.05$). The null hypothesis that age has no effect on TXA_2 concentrations was rejected. No significant differences in concentrations of TXA_2 due to the main effect of exercise were found. Thus, the null hypothesis remained tenable.

Resting TXA_2 Concentrations

In the present study, resting concentrations of TXA_2 between the two age groups were not significantly different. The hypothesis that resting plasma TXA_2 would not be different between the two age groups was accepted. This finding is consistent with results reported by Vericel et al. (1988); but, it does not concur with data reported by Reilly and FitzGerald (1986). Reilly and FitzGerald noted that resting urinary TXA_2 concentrations were 46.7% higher in

adults 50 to 88 years old when compared to adults 21 to 39 years old ($p < 0.005$). Vericel et al. (1988) reported that urinary TXA_2 concentrations at rest were two-fold higher in 78 to 94 year old adults when compared with 25 to 35 year old adults; but, the difference was not significant. Plasma TXA_2 was 48% greater in the older men, in the present study.

In contrast, Vericel et al. (1988) reported that serum concentrations of TXA_2 were two-fold greater in young adults when compared to older adults. The difference was statistically significant ($p < 0.05$). Reilly and FitzGerald (1986) found that serum TXA_2 was twelve percent higher in young adults. The apparently contrasting results associated with the analysis of serum TXA_2 may be due to platelet exposure to the high concentrations of thrombin released when whole blood is allowed to clot. Blood clotting is necessary for serum formation. Vericel et al. (1988) suggested that the availability of AA is depressed in older adults; and, when platelets from older subjects are exposed to high concentrations of thrombin there is less AA available for the formation of TXA_2. This hypothesis led these researchers to reason that urinary concentrations of TXA_2 were more representative of "basal" circulating concentrations than serum TXA_2. Vericel et al. (1988) also demonstrated that platelet TXA_2 formation was significantly greater in older adults following stimulation with low concentrations of thrombin. The 48% age-related difference in plasma

concentrations of TXA_2 found in the present study, which was similar to those reported by both Vericel et al. (1988) and Reilly and FitzGerald (1986), might be expected since plasma is extracted from whole blood without activation of the clotting mechanisms.

In vitro investigations of TXA_2 release following stimulation by AA (Chang, & Tai, 1983) and collagen (Giani, Masi, & Galli, 1985) indicate that platelets from older rats produce significantly more TXA_2 than younger rats. The results from these studies add additional support to the research involving human subjects.

The research by Reilly and FitzGerald (1986) suggest that older adults may have higher resting concentrations of TXA_2. Significant age-related differences were not found in the present study or by Vericel et al. (1988); however, the direction and the magnitude of difference found in these studies are in agreement with Reilly and FitzGerald's findings. The results from research involving animal models concur with the research conducted on humans.

Exercise and Recovery TXA_2 Concentrations

Concentrations of TXA_2 immediately following thirty minutes of exercise were similar for both the young and older men. The hypothesis that plasma TXA_2 would not be significantly different between the young and older men remained tenable. Other research pertaining to prostanoid concentrations in different age groups immediately following

exercise was not found; and, other groups studied consisted primarily of younger subjects.

Plasma TXA_2 immediately after exercise was not significantly different from resting concentrations. Other researchers have reported similar results (Carter, Ready, Singhroy, Duta, & Gerrard, 1989; Metha, Metha, & Horalek, 1983; Taniguchi, Furui, Yamauchi, & Sotobata, 1984; Todd, Goldfarb, & Boyer, 1990; Viinikka, Vuori, & Ylikorkala, 1984).

Carter et al. (1989) found that fifteen minutes of cycling at thirty percent of VO_2max was associated with a significant increase in TXA_2 concentrations. This response occurred only following mild exercise, and not in response to more intense exercise (Carter, Ready, Singhroy, Duta, and Gerrard, 1989). One potential problem with the study by Carter et al. (1989) pertains to the fact that an intravenous catheter was used to collect blood samples during the mild exercise and not during the more vigorous exercise. Furthermore, Todd et al. (1990) reported that plasma TXA_2 concentrations tended to be positively associated with exercise intensity in a study in which a catheter was used. A catheter may cause damage to the endothelial lining during exercise; and, the damage may be more pronounced at the higher exercise intensities, which may be associated with more vigorous movements. This hypothesis is partly supported by the research conducted by Mant et al. (1984). These

researchers reported that evidence of platelet activation (i.e. platelet factor four and beta thromboglobulin formation) was greater when blood samples from exercising subjects were collected from an indwelling catheter as opposed to venipuncture techniques (Mant, Kappagoda, & Quinlan, 1984). Platelet activation may be stimulated by TXA_2 formation.

The absence of exercise-induced alterations in TXA_2 concentrations immediately following exercise appear to be a consistent finding among the different research studies. Evidence indicating that circulating concentrations of TXA_2 may be increased during exercise is weak since indwelling catheters were used to collect data for these studies.

Plasma TXA_2 concentrations in the young men thirty minutes following exercise were similar to resting concentrations. However, TXA_2 concentrations among the older men were approximately twenty-nine percent higher when compared to their resting values; and, these concentrations were nearly two-fold greater than those found in the young men at the same observation time. The difference between the recovery values for the young and older men was not statistically significant; thus, the hypothesis that plasma TXA_2 would not be significantly different between the two age groups was accepted. These data suggest that in the absence of changes in PGI_2 older men may be more predisposed to platelet aggregation thirty minutes after exercise than at

rest or during exercise. The large degree of variability at each observation time indicates that this may not occur in all older subjects. This finding is unique to the present study as no other research pertaining to the effects of age and exercise on prostanoid concentrations was found.

The older men tended to display increases in TXA_2 concentrations thirty minutes after exercise, which may be partly related to exercise-induced increases in various agents known to stimulate platelet release of TXA_2. Vericel et al. (1988) demonstrated that platelet aggregation was greater in older adults when platelets were exposed to low concentrations of collagen (0.25 ug/ml) and epinephrine (2 uM). Both of these substances may induce platelet aggregation by stimulating TXA_2 release from platelets. The increased platelet aggregation found in their study was associated with increased TXA_2 production (Vericel et al., 1988). Furthermore, Hendra et al. (1988) reported that concentrations of both collagen and epinephrine needed to stimulate platelet aggregation following exercise were lower when compared to the concentrations needed prior to exercise. These two investigations indicate that older persons may be more predisposed to TXA_2 formation following platelet exposure to epinephrine and collagen and that platelet sensitivity to these substances increases with exercise.

Numerous studies have reported that epinephrine concentrations increase with exercise (Christensen, & Galbo,

1983). One might hypothesize that the increase in epinephrine during exercise may stimulate a greater increase in TXA_2 concentrations in older men when compared to young men. One problem with this hypothesis is that the increased TXA_2 concentrations were found thirty minutes following the completion of exercise, not immediately after exercise. Epinephrine concentrations return to pre-exercise concentrations within five to ten minutes following the cessation of exercise (Lehmann, Kapp, Himmelsbach, & Keul, 1982; Taniguchi, Furui, Yamauchi, & Sotobata, 1984). One might expect that TXA_2 concentrations would be elevated immediately following exercise as opposed to thirty minutes following exercise, unless other agents stimulate increases following exercise. Additionally, TXA_2 release initiated by epinephrine may be perpetuated by other agents. For example, TXA_2 may stimulate the release of additional TXA_2. This feed-forward mechanism may sustain increased release of TXA_2 after epinephrine concentrations have subsided. Results from the investigation conducted by Taniguchi et al. (1984) support this hypothesis. These researchers found that both epinephrine and TXA_2 concentrations immediately following exercise were elevated above resting concentrations. By six minutes after the cessation of exercise epinephrine had subsided to near resting concentrations, while TXA_2 remained elevated. Thirty minutes following exercise TXA_2

concentrations were still above resting values (Taniguchi, Furui, Yamauchi, & Sotobata, 1984).

No reports regarding the effect of acute exercise on circulating concentrations of collagen or platelet contact with collagenous tissue were found; however, it is plausible that contact between collagen and platelets may increase with exercise and that this interaction may continue after the cessation of exercise. Evidence indicates that older adults may be more susceptible to collagen-platelet interaction due to age-related increases in both collagen content within the vascular tissue (Barnes, 1988) and the number of collagen cross linkages (Nimni & Harkness, 1988).

The prevalence of both atherosclerosis and arteriosclerosis increases with age. Atherosclerosis is associated with the proliferation of platelets and other substances into the intimal layer of the vasculature. Upon proliferation into the vascular wall platelets release platelet derived growth factor which stimulates the growth of vascular tissue. This vascular repair mechanism provides a means for the development of plaque deposits when high concentrations of fat and cholesterol are present in the blood. When atherosclerotic plaque deposits are damaged, collagen fibers, which may be present in high concentrations within these plaque formations, come into contact with platelets (Barnes, 1985).

Arteriosclerosis is associated with a reduction in the elasticity of arterial tissue. The age-related loss of elasticity predisposes arterial linings to damage when capacitance demands suddenly change. For instance, when exercise is initiated and the demand for blood flow to the exercising muscles suddenly increases. Any resulting arterial damage may lead to increased contact between collagen and platelets (Guyton, 1986).

In addition to plaque and vascular tissue damage associated with alterations in blood flow, exercise-induced mechanical stresses may cause damage to plaque formations and sclerotic arteries. One must recognize that there is little evidence supporting this and other hypotheses regarding exercise mediated contact between platelets and collagenous tissue.

Circulating concentrations of TXA_2 immediately following thirty minutes of exercise were similar to resting values for both young and older men. No significant differences between the two groups were observed at this observation time. Plasma concentrations of TXA_2 thirty minutes after exercise tended to be higher among the older men when compared to the younger men, although the difference was not statistically significant. Thromboxane A_2 concentrations among the older group thirty minutes following exercise were twenty-nine percent greater than resting values. These data suggest that some older men may be more predisposed to platelet

aggregation following exercise when compared with younger men. The mechanisms mediating these apparent alterations in TXA_2 are unclear.

Lipid Profiles

Total serum cholesterol in the older group was twenty-three percent higher and significantly different from the young group. The use of total cholesterol as a constant covariate did not have a significant influence on the analyses of PGI_2/TXA_2 ratios and TXA_2 concentrations. Thus, the hypothesis that total cholesterol would not influence the effect of age and exercise on protanoid concentrations was accepted.

The impact of total cholesterol on prostanoids is difficult to evaluate since different cholesterol subfractions may have different effects. For example, HDL has been associated with an increase in PGI_2 synthesis; whereas, LDL has been associated with reduced PGI_2 production. (Beitz, & Forster, 1980). No other studies investigating the relationship between total serum cholesterol and prostanoid responses to acute exercise were found.

No between group differences were found for HDL. Therefore, HDL was not used as a covariate. Rauramaa et al. (1984) reported that an increase in HDL_2 subfraction following eight weeks of aerobic exercise training was positively correlated with increased plasma PGI_2

concentrations. These researchers also reported that HDL_3 subfractions declined significantly with exercise training. One weakness in the present study was that HDL subfractions were not evaluated; and, although total serum HDL was essentially the same for both groups, one does not know if there were any differences in the HDL subfractions.

There was not a significant difference between LDL values for the young and older men, although there was approximately a twenty percent difference between the two groups. A weak, but significant, inverse correlation was found between LDL and the thirty minute post-exercise PGI_2/TXA_2 ratios ($r = -0.41$; $p < 0.05$). Similar inverse correlations, which approached significance, were found between LDL and both resting ($r = -0.39$; $p = 0.059$) and immediate post-exercise ($r = -0.39$; $p = 0.060$) PGI_2/TXA_2 ratios. The age group-related main effect differences between PGI_2/TXA_2 ratios were eliminated when LDL was used as a covariate. The addition of LDL as a covariate did not make a significant contribution to the analysis. The p-values representing the effect of the between subject factor, age-group was ($p = 0.101$), and the regression factor, LDL was ($p = 0.115$). The hypothesis that there would not be a significant difference between PGI_2/TXA_2 ratios during rest, exercise or thirty minutes following exercise when LDL was used as a covariate remained tenable; but, these values suggest that LDL may be related to PGI_2/TXA_2 ratios. The small sample size and the large degree

of variability among prostanoid concentrations in both groups and among LDL in the older group probably contributed to the low r-values and the lack of significance.

These data seem to suggest that individuals with high serum LDL concentrations may have lower PGI_2/TXA_2 ratios both at rest and following exercise, regardless of their age. Research by Beitz and Forster (1980) indicated that serum LDL from humans inhibited in vitro PGI_2 synthesis. Their results tend to support the notion that LDL may also alter the PGI_2/TXA_2 ratio. It is difficult to ascertain whether or not LDL directly altered PGI_2 concentrations in the present study. According to Vericel et al. (1988) age is apparently positively related to plasma PGI_2; whereas, LDL has been inversely associated with PGI_2 synthesis (Beitz, & Forster, 1980). Resting PGI_2 concentrations in the present study were approximately sixteen percent higher in the older men. Immediately following exercise and thirty minutes later PGI_2 concentrations were essentially the same in both groups with neither group experiencing any significant exercise-induced alterations. One might speculate that under resting conditions the influence age had on plasma PGI_2 was predominant, compared to LDL's effect on PGI_2. Following exercise there were differences in PGI_2 concentrations. Assessment of the potentially confounding influences age and LDL concentrations have on circulating concentrations of PGI_2 and TXA_2 is warranted. Additionally, one might measure LDL

concentrations during and after acute exercise in order to more precisely determine the role of LDL, although there is little evidence to suggest that acute exercise has an effect on LDL. The present study provides sufficient evidence to support further investigation of the influence of LDL on prostanoid concentrations during and after exercise.

Dietary Characteristics

Mean daily intake of calories, fat and saturated fat intake were similar for both groups in the present study. Mean daily intake of linoleic acid expressed as a percentage of the mean daily intake of calories was not significantly different between the two groups. The values were 5.74 $\pm$ 0.07% and 4.98 $\pm$ 0.02% for the young and older group, respectively. DuPont (1987) recommended that six to ten percent of total daily energy intake come from linoleic acid to insure that adequate substrate is available for prostanoid synthesis; and, the amount of linoleic acid needed is inversely related to P/S ratio. Intake of linoleic acid by the two groups in the present study approached the lower end of the requirements, but did not meet the recommendations made by DuPont (1987). The P/S ratio within each group equaled or exceeded the recommended dietary allowance of 1.0. The P/S ratios were 1.66 $\pm$ .36 and 1.00 $\pm$ .36 for the young men and older men, respectively. The linoleic acid intake requirements for both the young and older groups were near the low end of the percentage of total energy intake range.

Whereas dietary linoleic acid values found in the present study suggest that intake in both groups may have been slightly deficient, plasma concentrations of both PGI_2 and TXA_2 were within the ranges reported by other researchers (Metha, Metha, & Horalek, 1983; Taniguchi, Furui, Yamauchi, & Sotobata, 1984).

One weakness with the results pertaining to linoleic acid was that concentrations of this prostanoid substrate were estimated via dietary food records. Neither the actual intake nor circulating concentrations of linoleic acid were measured.

A significant difference in the mean daily intake of polyunsaturated fat was found between the two groups ($p < 0.05$). Polyunsaturated fat intake was approximately 48.1% higher in the young group. A weak, but significant, correlation was found between polyunsaturated fat intake and resting PGI_2 concentrations. Polyunsaturated fat intake was not significantly related to PGI_2 at any other observation time. Polyunsaturated fat was used a covariate since a significant difference was found between the two groups. Addition of polyunsaturated fat as covariate did not make a significant contribution to the analysis.

The difference between P/S ratios found in each group were not statistically significant. The ratio among the young men was 66% greater than that found among the older

men, but the mean values in the young group was marked by a large degree of variability.

The dietary data did not provide sufficient information to help clarify the unique findings in the present study, although a significant difference was found between group intake of polyunsaturated fat. Dietary information might be useful in future research, particularly if larger sample sizes are used.

CHAPTER VI

CONCLUSIONS AND RECOMMENDATIONS

The results of the present study suggest that thirty minutes following exercise, some older men may be more predisposed to platelet aggregation due to a decline in the PGI_2/TXA_2 ratio. This exercise-induced response was characterized by plasma TXA_2 concentrations that were nearly two-fold greater in the older men compared to the younger men. The lack of statistically significant differences indicates that further investigation is needed.

The large degree of variability present in some of prostanoid data contributed in part to the fact that no statistically significant differences were obtained. Some of the variability among the prostanoid values may have been related to the relatively wide range of fitness categories represented by the subjects. Subjects representing more homogenous fitness categories might help eliminate some of the variation among the prostanoid data. A greater sample size may also help increase the chances of obtaining statistically significant differences.

Total cholesterol and polyunsaturated fat intake were significantly different between the two groups; and, LDL and P/S ratios approached statistically significant differences. The use of total cholesterol, LDL, polyunsaturated fat and P/S ratios as covariates did not alter the results for the

statistical analysis of the effect of age and exercise on prostanoid concentrations. Serum HDL, linoleic acid intake and saturated fat intake were similar between both groups. Evaluation of each of these parameters should be included in future research since other researchers have reported that they may help to clarify differences found when evaluating circulating concentrations of PGI_2 and TXA_2.

Other research suggest that individuals older than those involved in the present study may experience greater age-related increases in circulating prostanoid concentrations. Therefore, future research designed to investigate the effect of age on TXA_2 and PGI_2 concentrations should involve subjects older than the one's included in this study.

Verification of platelet aggregation and evidence of platelet activation in the presence of a decline in PGI_2/TXA_2 ratios would add strength to the results reported in the present study. A number of techniques allowing estimation of in vivo platelet aggregation exist; and, measurement of beta-thromboglobulin or platelet factor IV would provide evidence of platelet activation. If exercise-induced increases in TXA_2 are associated with increased platelet aggregation or activation then the specific TXA_2 synthase blocker, imidazole, should be used to substantiate this relationship.

Future research should involve the collection of samples at several different times during recovery from exercise to

determine when TXA_2 concentrations peak following exercise. The investigation of the effects of different durations and intensities of exercise may help clarify which exercise conditions are associated with the greatest alterations in prostanoid concentrations. Investigations conducted by Rauramaa et al. (1984) suggest that exercise training may lead to favorable changes in prostanoid synthesis. Future research should investigate the effects of exercise training on circulating prostanoids in older adults.

The effect of age and exercise on PGI_2/TXA_2 ratios were investigated in only male subjects in the present study. Female subjects were not included because lipid profiles between men and women have been found to be significantly different; thus, the inclusion of women in the present study may have presented an additional confounding factor influencing the results. Future research should involve comparisons between male and female subjects.

Further investigation of the interaction between age, exercise, lipid profiles and diet and the effect of these factors on prostanoids may provide valuable information necessary for identifying mechanisms associated with platelet aggregation. Such research may also be useful in identifying persons at higher risk of exercise-induced thrombosis formation.

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APPENDIX A

(Informed Consent: Young Adults)

CONSENT FORM FOR HUMAN SUBJECTS (young adults)

THE UNIVERSITY OF NORTH CAROLINA AT GREENSBORO
SCHOOL OF HEALTH, PHYSICAL EDUCATION AND RECREATION

Subject's Name: _____

Project Title: Effect of Age and Acute Exercise on Circulating Concentrations of Prostacyclin and Thromboxane A_2 in Adult Males

Project Coordinator: Mikel Kent Todd, Doctoral Candidate
Department of Exercise and Sport Sciences

I understand that the purpose of this study is to determine if there is a difference between the circulating ratio of prostacyclin and thromboxane A_2, two substances found in the blood that have an important role in platelet aggregation. Whereas this study may not directly benefit me, it may advance knowledge in the field of exercise physiology and medicine.

I understand that taking aspirin, ibuprofen (Advil, Medipren, Motrin or Nuprin), indomethacin (Indocin) or similar medications during this study may interfere with the results of this study. Thus, I agree not to take any of these medications for 1 week prior to the maximal exercise test or at any time prior to completion of my participation in the study. If I am prescribed any of these medications during my participation in this test I will immediately notify the project coordinator. I understand that if I do need to take an analgesic (pain reliever) while I am a participant that I may take one containing acetaminophen (Tylenol).

During the study I will report to the Exercise Physiology Laboratory on the UNC-Greensboro campus (HPERD Building #240) for 3 visits. During the first visit I will be informed of the procedures involved in the study; and, I will be asked to answer questions regarding my medical history. I understand that between the first visit and the time that I complete the study I must use Mazola margarine and cooking oil in place of any other margarines or oils I currently use.

During the second visit I will perform a maximal exercise stress test on a motorized treadmill. I understand that during this test electrodes will be attached to my torso so that my cardiac response to the exercise may be monitored. I understand that it may be necessary for the researcher to shave hair from my chest to properly attach the electrodes.

I understand that during and/or after this test I may become dizzy, tired or weak as a result of fatigue. I understand that the results from this test will be used to determine the intensity levels for the sub-maximal tests. My performance on this test will be explained to me afterwards.

I understand that there is a rare possibility that I may suffer a heart attack (<3 in 10,000) or death (<1 in 10,000) as a result of the maximal exercise test, but that the researchers will minimize such risk by pre-liminary screening and continuous monitoring throughout the test.

I understand that between the second and third visit I will be asked to complete a 4-day dietary food record. I understand that within 2 weeks I will return to the lab for 1 more visit to perform a sub-maximal exercise test at 70% to 75% of my maximal oxygen consumption.

I understand that each sub-maximal test will be conducted as follows. I will enter the lab after an overnight fast. Upon entering the lab, I will be asked to rest in the supine position for 15 minutes. After this rest period 20 milliliters of blood will be taken from and antecubital vein located near the elbow region of my arm. The blood will be taken using sterile venipuncture techniques. I understand that the venipuncture may be slightly painful and that there is a slight risk of infection at the insertion site. I understand that the researchers will take precautions to prevent infection. To help stop bleeding after the needle is removed from my vein I will apply pressure with gauze to the venipuncture site until the researcher tells me to stop. After the blood sample has been taken, I will remain lying down while electrodes are attached to my torso. I will begin a 30 minute run on the treadmill at the intensity mentioned above. Following the 30 minute run I will cool down on the treadmill until my heart rate has dropped to below 110 beats per minute. Within 5 minutes after the 30 minute run another 20 milliliter blood sample will be taken. After I have sufficiently cooled down I will rest for 30 minutes. At the end of 30 minutes another 20 milliliter blood sample will be taken.

I confirm that my participation in this study is voluntary, and that no coercion has been used to obtain my cooperation. I also understand that I can terminate my participation in this study at any time. I understand that all information obtained in this study will remain confidential and anonymous. I understand that a summary of the results of the study will be made available to me, per my request, after the completion of this study.

I confirm that I have been informed of the procedures that will be used in this study. I understand what is required of me as a subject and the risk involved. I agree that any questions I have regarding this study and the procedures have been answered to my satisfaction; and, I wish to give my voluntary cooperation as a participant.

Questions: _____

_____

_____

Responses: _____

_____

_____

_____ _____

Signature of Subject Phone Number

_____ _____

Address Date

_____ _____

Witness

APPENDIX B

(Informed Consent: Older Adults)

CONSENT FORM FOR HUMAN SUBJECTS (older adults)

THE UNIVERSITY OF NORTH CAROLINA AT GREENSBORO
SCHOOL OF HEALTH, PHYSICAL EDUCATION AND RECREATION

Subject's Name: _____

Project Title: Effect of Age and Acute Exercise on Circulating Concentrations of Prostacyclin and Thromboxane A_2 in Adult Males

Project Coordinator: Mikel Kent Todd, Doctoral Candidate
Department of Exercise and Sport Science

I understand that the purpose of this study is to determine if there is a difference between the circulating ratio of prostacyclin and thromboxane A_2, two substances found in the blood that have an important role in platelet aggregation. Whereas this study may not directly benefit me, it may advance knowledge in the field of exercise physiology and medicine.

I understand that taking aspirin, ibuprofen (Advil, Medipren, Motrin or Nuprin), indomethacin (Indocin) or similar medications during this study may interfere with the results of this study. I agree not to take any of these medications for 1 week prior to the maximal exercise test or at any time prior to completion of my participation in the study. If I am prescribed any of these medications during my participation in this test I will notify the project coordinator. I understand that if I need to take an analgesic (pain reliever) while I am a participant that I may take one containing acetaminophen (Tylenol).

During the study, I will report to the Student Health Center at UNC-Greensboro (Room #207) or the Exercise Physiology Laboratory in the HPRD Building (Room #240) for 3 visits. I understand that the researcher will inform me about the location where I am supposed to report. During the first visit I will be informed of the procedures involved in the study; and, I will be asked to answer questions regarding my medical history. I understand that between the first visit and the time that I complete the study I must use Mazola margarine and cooking oil in place of any other margarines or oils I currently use. Also, between the first and second visit I must meet with my physician. I understand that I must ask my physician to for: (1) written permission supporting my participation in this study and (2) the release of any pertinent medical information to the UNC-Greensboro Student Health Center. Documents regarding these issues will

be given to me during the first visit and must be returned to the project coordinator before I further participate in this study. I also understand that a list of "Contraindications for Exercise Testing" will be given to me to give to my physician, and that my physician will also be asked to verify that he or she was provided with this information.

During the second visit I will perform a maximal exercise stress test on a motorized treadmill. I understand that during the test electrodes will be attached to my torso so that my cardiac response to the exercise may be monitored. I understand that it may be necessary for the researcher to shave hair from my chest to properly attach the electrodes. I understand that during and/or after this test I may become dizzy, tired or weak as a result of fatigue. I understand that the results from this test will be used to determine the intensity levels for the sub-maximal tests. My performance on this test will be explained to me afterwards.

I understand that there is a rare possibility that I may suffer a heart attack (<3 in 10,000) or death (<1 in 10,000) as a result of the maximal exercise test, but that the researchers will minimize such risk by preliminary screening and continuous monitoring throughout the test. I understand that the maximal test will be conducted either in the Student Health Center, where a physician is on the premises, or the Exercise Physiology Laboratory, where a physician will be directly supervising the test. If the test is conducted in the Exercise Physiology Laboratory, the Emergency Medical Services will be notified in advance about the time, location and nature of the test. I understand that if I have a heart attack during the test, cardiopulmonary resuscitation (CPR) or defibrillation may used for life support. I understand that the life sustaining cardiac medications will not be administered until the Emergency Medical Services arrive, and that the risk of death from a heart attack may be greater during the maximal exercise testing at the Exercise Physiology Laboratory than the Student Health Center, hospital, or a doctor's office. I am aware of the increased risk and accept it as part of my participation. I have been assured that American College of Sports Medicine Guidelines for Exercise Testing and Prescription will be followed during all phases of this study.

I understand that between the second and third visit I will complete a 4-day dietary food record. I understand that within 2 weeks, I will return for 1 more visit to perform a sub-maximal exercise test at 70% to 75% of my maximal oxygen consumption which was calculated from the results of my maximal exercise test.

I understand that each sub-maximal test will be conducted as follows. I will enter the lab after an overnight fast. Upon entering the lab, I will be asked to rest in the supine position for 15 minutes. After this rest period 20 milliliters of blood will be taken from and antecubital vein located near the elbow region of my arm. Blood will be taken using sterile venipuncture techniques. I understand that the venipuncture may be slightly painful and that there is a slight risk of infection at the insertion site. I understand that the researchers will take precautions to prevent infection. To help stop bleeding after the needle is removed from my vein, I will apply pressure with gauze to the venipuncture site until the researcher tells me to stop. After the blood sample has been taken, I will remain lying down while electrodes are attached to my torso. I will begin a 30 minute run on the treadmill at 70% to 75% of my maximal oxygen consumption. Following the 30 minute run, I will cool down on the treadmill until my heart rate has dropped to below 110 beats per minute. Within 5 minutes after the 30 minute run another 20 milliliter blood sample will be taken. After I have sufficiently cooled down, I will rest for 30 minutes. At the end of 30 minutes another 20 milliliter blood sample will be taken.

I confirm that my participation in this study is voluntary, and that no coercion has been used to obtain my cooperation. I also understand that I can terminate my participation in this study at anytime. I understand that all information obtained in this study will remain confidential and anonymous. I understand that a summary of the results of the study will be made available to me, per my request, after the completion of this study.

I confirm that I have been informed of the procedures that will be used in this study. I understand what is required of me as a subject and the risk involved. I agree that any questions I have regarding this study and the procedures have been answered to my satisfaction; and, I wish to give my voluntary cooperation as a participant.

Questions: _____

_____

_____

Responses: _____

_____

_____

_____ _____

Signature of Subject | Phone Number

_____ _____

Address | Date

_____ _____

Witness

APPENDIX C

(Medical History)

Medical History

History of Cardiovascular Disease:

Heart attack	_____
Chest discomfort	_____
Heart murmurs	_____
Extra or skipped beats	_____
Congenital heart disease	_____
Cardiac surgery	_____
Peripheral Vascular Disease	_____
Rheumatic Fever	_____
Phelbitis, emoboli	_____
Unusual shortness of breath	_____

Major Coronary Risk Factors:

History of high blood pressure (145/95)	_____
Elevated TC/HDL ratio	_____
Smoking	_____
Abnormal resting ECG	_____
Family history of CHD prior to age 50	_____
Diabetes	_____

Other Factors:

Ankle swelling	_____
Pulmonary disease (asthma, emphysema and bronchitis)	_____
Lightheadedness or fainting	_____
Emotional disorders	_____
Stroke	_____
Recent illness or hospitalization	_____
Orthopedic problems	_____
Caffine intake (including colas)	_____
Dieting	_____

Exercise History: (Type/Intensity/Duration/Frequency)

Medications:

APPENDIX D

(Physician's Disclosure and Approval Statement)

Physician's Disclosure and Approval for Participation

I, _____, based on my professional assessment, hereby agree that, _____, otherwise known as the research subject, can safely participate in the dissertation research to be conducted by Mikel Kent Todd at the Student Health Center at the University of North Carolina at Greensboro. I confirm that as part of my assessment I have reviewed the research subject's medical history. I understand that as a participant in this study the research subject will participate in both a maximal exercise stress test and a sub-maximal exercise session. I understand that both exercise sessions will be performed an a motorized treadmill with continuous 12-lead electorcardiographic monitoring. I understand that a physician will be in the Student Health Center building during the testing, that the physician will be alerted to the fact that the testing is underway and that the physician will be available for any emergency situations that may arise as a result of the testing. I understand the sub-maximal exercise session will be 30 minutes in duration and that the research subject will exercise at 70 to 75% of his maximal oxygen consumption. I confirm that (1) I am aware of the risk associated with exercise testing (2) the research subject has provided me with the list of "Contraindications for Exercise Testing" and a resting 12-lead electrocardiograph and (3) I have reviewed these documents.

Right to Release Medical Information

I, _____, have granted the physician, _____, permission to provide the Student Health Center physicians at the University of North Carolina at Greensboro with any pertinent medical information necessary to safely treat me during any medical emergency arising as a result of my participation in the exercise testing portion of the dissertation research conducted by Mikel Kent Todd.

I, _____, otherwise known as the subject's physician, agree to provide the Student Health Center physicians at the University of North Carolina at Greensboro with any pertinent medical information necessary to safely treat, _____, the research subject, during any medical emergency arising as a result of participation in the dissertation research conducted by Mikel Kent Todd.

physician's signature	date
research subject's signature	date
physician's office phone #	other day time phone #

APPENDIX E

(Contraindications to Exercise Testing)

Contraindications to Exercise Testing

"There are certain individuals for whom the risks of testing outweigh the potential benefits. These individuals should not be tested. There are other individuals whose medical conditions increase the risk of testing. It is important in these circumstances for the test administrator to weigh carefully the anticipated benefits and determine that these outweigh the risk."*

Table I. DEFINITE CONTRAINDICATIONS TO EXERCISE TESTING

1.	Recent acute myocardial infarction
2.	Unstable angina
3.	Uncontrolled ventricular dysrhythmia
4.	Uncontrolled atrial dysrhythmia which compromises cardiac function
5.	Congestive heart failure
6.	Severe aortic stenosis
7.	Suspected or known dissecting aneurysm
8.	Active or suspected myocarditis
9.	Thrombophlebitis or intracardiac thrombi
10.	Recent systemic or pulmonary embolus
11.	Acute infection
12.	Third degree heart block
13.	Significant emotional distress (psychosis)
14.	Recent significant change in the resting ECG
15.	Acute pericarditis

Table II. RELATIVE CONTRAINDICATIONS TO EXERCISE TESTING

1.	Resting diastolic blood pressure over 120 mm Hg or resting systolic blood pressure over 200 mm Hg
2.	Moderate valvular heart disease
3.	Digitalis or other drug effect
4.	Electrolyte abnormalities
5.	Fixed rate artificial pacemaker
6.	Frequent of complex ventricular irritability
7.	Ventricular aneurysm
8.	Cardiomyopathy including hypertrophic cardiomyopathy
9.	Uncontrolled metabolic disease (diabetes, thyrotoxicosis, myxedema, etc.)
10.	Any serious systemic disorder (mononucleosis, hepatitis, etc.)
11.	Neuromuscular, musculoskeletal, or rheumatoid disorders which would make exercise dangerous or difficult

*From the Guidelines for Exercise Testing and Exercise Prescription, American College of Sports Medicine. (1986).

APPENDIX F

(Maximal Exercise Test Record)

MAXIMAL EXERCISE TEST RECORD

Has the subject provided informed consent? yes no

Has the physician approved particiaption? yes no

Name: _____ Date: _____

Age: _____ Age predicted maximal heart rate: _____

Stage (min):	spd/grd	HR	BP	VO_2	Remarks:
Rest	0				
I ( )					
II ( )					
III ( )					
IV ( )					
V ( )					
VI ( )					
VII ( )					
IPE					
Recovery (2)					
Recovery (5)					

Comments:

APPENDIX G

(Four-Day Dietary Food Record)

Subject's Name:_____

SUMMARY OF HOW TO RECORD FOOD PORTIONS

Record in Ounces: (1 cup = 8 ounces; 1 pint = 16 ounces)

Beverages - all types, including alcoholic

Record in Cups or Servings: (small or large serving)

Potatoes, rice, etc.
Fruits, vegetables (cooked or canned)
Soups, cereals, casserole dishes

Record in Teaspoons or Tablespoons:

(3 teaspoons = 1 tablespoon)

Jelly, jam, sugar, syrup
Salad dressing, sauces, gravies
Butter, margarine (may list as no. of pats)

Record by Number and Size:

Bread, rolls, crackers
Raw fruits and vegetables
Meat cuts, chicken, frankfurters, shellfish
Snack items - nuts, cookies, candy, etc.

Record by Servings: (small or large serving)

Pie, cake, coffee cake
Pizza

FOOD PREPARATION NOTES

To be completed by the person preparing meals at home.

1. Kind of cooking fat used: _____ (Type) _____ (Brand Name)

2. Salad dressing used: _____ (Type) _____ (Brand Name)

3. Was fat used to cook vegetables? Yes No _____ (Type)

4. Spread used on bread: _____ (Type) _____ (Brand Name)

5. Was milk used in cooking? Yes No % fat _____

6. Fat used in baking: ______________ Type ______________ Brand Name

Subject's Name: ______________________________

Day: ______________________ Date: ______________________

Was this an unusual day? Yes No

If yes, please explain: ______________________________

____

Time	At Home or Out	What You Ate and How Much

APPENDIX H

(Sub-maximal Exercise Session Record)

SUB-MAXIMAL EXERCISE SESSION RECORD

Name: _____ Date: _____

Age: _____ Target Heart Rate Range: _____

VO_2max: _____ (ml/kg/min) VO_2 range: _____

Time (min):	spd/grd	HR	BP	VO_2	Remarks:
Rest	0				
5					
10					
15					
20					
25					
30					
Recovery (2)					
Recovery (5)					

Comments:

APPENDIX I

(Raw and Computed Data; with Codes)

Raw and Computed Data Codes

ID:	subject identification code
Age:	age (years).
Weight:	weight (lbs).
RHR:	resting heart rate; lowest heart rate recorded (bpm).
MHR:	peak heart rate during maximal exercise test (bpm).
HR5:	5 minute heart rate during sub-maximal exercise (bpm).
HR10:	10 minute heart rate during sub-maximal exercise (bpm).
HR15	15 minute heart rate during sub-maximal exercise (bpm).
HR20:	20 minute heart rate during sub-maximal exercise (bpm).
HR25:	25 minute heart rate during sub-maximal exercise (bpm).
HR30:	30 minute heart rate during sub-maximal exercise (bpm).
VO_2max:	maximal oxygen consumption (ml/kg/min).
$VO_2$5:	mean VO_2 recorded between 5 & 10 min during sub-maximal exercise (ml/kg/min).
$VO_2$20:	mean VO_2 recorded between 20 & 25 min during sub-maximal exercise (ml/kg/min).
%$VO_2$5:	%$VO_2$5 = $VO_2$5 / VO_2max
%$VO_2$20:	%$VO_2$20 = $VO_2$20 / VO_2max
TC:	total cholesterol (mg/dl).
HDL:	high density lipoprotein (mg/dl).
LDL:	low density lipoprotein (mg/dl).
Hct1:	resting hematocrit.
Hct2:	exercise hematocrit.
Hct3:	recovery hematocrit.
Hgb1:	resting hemoglobin (g/dl).
Hgb2:	exercise hemoglobin (g/dl).
Hgb3:	recovery hemoglobin (g/dl).
PC1:	resting platelet count (x 1000/ml).
APC2:	exercise platelet count adjusted for hemoconcentration (x 1000/ml).
APC3:	recovery platelet count adjusted for hemoconcentration (x 1000/ml).
PVB:	resting plasma volume.
%PVE:	exercise plasma volume as a percentage of resting plasma volume.
%PVR:	recovery plasma volume as a percentage of resting plasma volume.
PGI1:	resting 6-keto-Prostaglandin F_1 .
PGI2:	exercise 6-keto-Prostaglandin F_1 .
APGI2:	APGI2 = PGI2 * (%PVE)

Raw and Computed Data Codes (continued).

PGI3:	recovery 6-keto-Ptostaglandin F_1 .
APGI3:	APGI3 = PGI3 * (%PVR)
TXA1:	resting Thromboxane B_2 .
TXA2:	exercise Thromboxane B_2 .
ATXA2:	ATXA2 = TXA2 * (%PVE)
ApcTXA2:	ApcTXA2 = ATXA2 * (APC2 / PC1)
ATXA3:	ATXA = TXA * (%PVR)
ApcTXA3:	ApcTXA2 = ATXA3 * (APC3 / PC1)
B-ratio:	B-ratio = PGI1 / TXA1
E-ratio:	E-ratio = APGI2 / ATXA2
Epc-rat:	Epc-rat = APGI2 / ApcTXA2
R-ratio:	R-ratio = PGI3 / ATXA3
Rpc-rat:	Rpc-rat = PGI3 / ApcTXA3
ADFG:	Average Daily Intake of Fat in Grams
ADC:	Average Daily Intake of Calories
ADSFG:	Average Daily Intake of Saturated Fat in Grams
ADPFG:	Average Daily Intake of Polyunsaturated Fat in Grams
PSR:	Polyunsaturated/Saturated Fat Ratio
ADLA:	Average Daily Intake of Lenoleic Acid expressed as a percentage of ADC.

Raw and Computed Data (young adults)

ID	Y2	Y3	Y4	Y5	Y6	Y7	Y8	Y9	Y10	Y11
Age	25	25	29	25	31	26	29	25	28	32
Weight	148	136	150	135	131	148	197	163	155	152
RHR	104	69	56	66	76	67	--	91	74	86
MHR	206	199	196	192	199	200	185	---	190	191
HR5	---	145	156	158	138	160	135	165	---	163
HR10	185	150	156	158	150	162	140	170	150	168
HR15	185	155	156	166	153	165	142	173	153	163
HR20	185	155	156	170	153	170	152	176	156	168
HR25	197	162	158	170	162	173	151	180	160	170
HR30	---	165	162	170	165	174	---	180	163	173
VO_2max	50.5	57.3	52.6	58.1	66.8	41.1	60.2	54.5	55.7	41.3
$VO_2$5	36.1	42.0	37.0	42.0	38.2	29.6	41.6	39.8	36.5	31.2
$VO_2$20	37.5	42.3	37.8	43.5	46.0	31.2	43.7	40.5	39.8	31.0
%$VO_2$5	.72	.73	.70	.72	.57	.72	.69	.73	.65	.76
%$VO_2$20	.74	.74	.72	.75	.69	.77	.73	.74	.71	.75
TC	196	137	186	197	165	218	150	135	162	182
HDL	49	42	50	57	39	92	48	42	58	35
LDL	133	80	119	126	114	114	88	82	91	108
Hct1	.461	.433	.417	.457	.466	.455	.499	.449	.456	.516
Hct2	.468	.461	.413	.467	.502	.478	.522	.467	.460	.533
Hct3	.458	.440	.380	.466	.462	.431	.491	.441	.434	.413
Hgb1	16.1	11.0	15.2	16.0	12.0	16.2	13.4	11.0	15.6	16.4
Hgb2	16.7	11.9	15.0	16.6	12.5	16.7	13.6	11.4	15.3	16.5
Hgb3	15.9	11.0	13.0	15.6	12.0	15.2	14.3	10.7	15.1	16.4

Raw and Computed Data (young adults) cont.

ID	Y2	Y3	Y4	Y5	Y6	Y7	Y8	Y9	Y10	Y11
PC1	272	276	210	261	325	292	250	228	321	291
APC2	277	255	272	271	332	314	275	261	361	397
APC3	269	273	228	232	338	305	214	202	346	283
PVB	53.9	56.7	58.3	54.3	53.4	54.5	50.1	55.1	54.4	48.4
%PVE	0.95	0.88	1.02	0.95	0.89	0.95	0.94	0.94	1.01	0.96
%PVR	1.02	0.99	1.24	1.01	1.01	1.11	0.96	1.05	1.08	1.01
PGI1	400	476	380	240	164	112	140	920	460	384
PGI2	459	880	464	344	256	284	220	540	620	448
APGI2	438	771	437	326	228	269	207	505	627	430
PGI3	374	336	272	252	216	280	364	352	376	520
APGI3	381	332	338	255	218	311	348	368	404	523
TXA1	82	52	30	18	30	54	42	102	40	82
TXA2	152	25	29	136	24	22	36	34	30	54
ATXA2	145	22	30	129	21	21	34	32	30	52
ApcTXA2	148	20	38	134	22	22	37	36	34	71
TXA3	82	92	38	52	20	32	68	68	50	76
ATXA3	84	91	47	53	20	36	65	71	54	76
ApcTXA3	83	90	51	48	21	37	56	63	58	74
B-ratio	4.8	9.2	12.7	13.3	5.5	2.1	3.3	9.0	11.5	4.7
E-ratio	3.0	35.2	16.0	2.5	10.7	12.9	6.1	15.9	20.7	8.3
Epc-rat	3.0	38.1	12.3	2.4	10.5	12.0	5.5	13.9	18.3	6.1
R-ratio	4.6	3.7	7.2	4.9	10.8	8.8	5.4	5.2	7.5	6.8
Rpc-rat	4.6	3.7	6.6	5.5	10.4	8.4	6.2	5.9	7.0	7.0

Raw and Computed Data (young adults) cont.

ID	Y2	Y3	Y4	Y5	Y6	Y7	Y8	Y9	Y10	Y11
ADFG	80	74	97	131	57	58	110	127	59	61
ADC	3101	2195	2739	2485	1542	2185	4069	3145	2281	1957
ADSFG	22.2	30.2	14.8	58.9	26.3	19.1	32.2	32.5	16.0	12.3
ADPFG	30.8	15.8	48.7	35.3	16.2	27.8	41.0	51.5	18.7	44.0
ADLA	0.02	0.03	0.05	0.07	0.02	0.03	0.04	0.03	0.03	0.06

Raw and Computed Data (older adults)

ID	O1	O2	O3	O4	O5	O6	O7	O8	O9	O10
Age	55	62	55	55	50	62	52	52	58	60
Weight	202	150	201	158	204	144	176	176	167	194
RHR	68	58	94	53	74	62	53	62	66	70
MHR	162	140	185	175	174	158	170	177	177	163
HR5	128	123	132	---	---	128	140	147	132	119
HR10	131	122	139	---	---	123	148	148	138	129
HR15	134	122	152	---	---	119	148	153	142	130
HR20	134	123	153	---	---	120	151	155	150	133
HR25	138	122	155	---	---	126	152	158	152	137
HR30	139	122	162	---	---	122	152	156	153	137
VO_2max	40.3	45.8	30.2	52.3	38.8	44.7	39.3	40.1	47.1	28.5
$VO_2$5	30.2	34.7	16.9	37.8	26.5	31.7	39.9	30.2	30.8	19.4
$VO_2$20	31.3	37.0	22.1	----	29.1	30.2	30.4	30.4	33.2	21.4
%$VO_2$5	.75	.76	.56	.72	.68	.71	.76	.75	.65	.68
%$VO_2$20	.78	.81	.73	---	.75	.68	.77	.76	.71	.75
TC	223	192	244	228	285	227	187	173	164	232
HDL	56	62	--	70	38	64	56	43	52	42
LDL	115	120	---	141	195	148	121	109	75	152
Hct1	.467	.443	.455	.433	.490	.443	.475	.476	.486	.472
Hct2	.482	.469	.446	.404	.510	.418	.476	.479	.498	.488
Hct3	.459	.448	.437	.427	.432	.429	.438	.441	.470	.458
Hgb1	16.6	16.3	15.0	15.0	15.0	16.1	16.4	18.7	15.3	14.6
Hgb2	17.8	16.1	15.0	15.5	17.4	16.0	17.2	18.0	14.8	15.7
Hgb3	15.2	14.0	14.3	15.2	17.6	14.2	16.4	15.9	15.8	16.1

Raw and Computed Data (older adults) cont.

ID	O1	O2	O3	O4	O5	O6	O7	O8	O9	O10
PC1	241	219	266	317	270	212	286	293	275	257
APC2	280	310	346	436	307	229	283	361	315	308
APC3	288	272	333	432	345	228	282	377	276	225
PVB	53.3	55.7	54.5	56.7	51.0	55.7	52.5	52.4	51.4	52.8
%PVE	0.91	0.97	1.01	1.01	0.83	1.05	0.95	1.03	1.01	0.90
%PVR	1.11	1.15	1.08	1.00	0.95	1.16	1.09	1.25	1.00	0.93
PGI1	204	808	392	168	196	304	336	448	820	848
PGI2	376	480	380	500	256	396	352	360	420	400
APGI2	342	464	386	507	212	415	336	370	425	360
PGI3	228	728	320	432	248	384	232	116	488	528
APGI3	253	840	346	431	236	444	253	145	488	490
TXA1	34	96	35	82	60	80	56	52	100	212
TXA2	25	74	34	64	46	122	34	56	114	72
ATXA2	23	71	34	65	38	128	32	58	115	65
ApcTXA2	26	101	45	89	43	138	32	71	132	78
TXA3	38	40	163	68	82	64	76	38	100	164
ATXA3	42	46	173	68	78	74	83	48	100	152
ApcTXA3	51	57	216	93	100	80	82	61	100	133
B-ratio	6.0	8.4	11.2	2.1	3.3	3.8	6.0	8.6	8.2	4.0
E-ratio	15.0	6.5	11.2	7.8	5.6	3.3	10.4	6.4	3.7	5.6
Epc-rat	12.9	4.6	8.6	5.7	4.9	3.0	10.5	5.2	3.2	4.6
R-ratio	6.0	18.2	2.0	6.4	3.0	6.0	3.1	3.1	4.9	3.2
Rpc-rat	5.0	14.7	1.6	4.7	2.4	5.6	3.1	2.4	4.9	3.7

Raw and Computed Data (older adults) cont.

ID	O1	O2	O3	O4	O5	O6	O7	O8	O9	O10
ADFG	65	49	103	142	101	82	69	48	46	97
ADC	2287	2534	3002	2857	2298	3605	1717	1875	1682	2312
ADSFG	19.4	14.4	33.6	26.6	24.5	23.4	18.5	15.4	13.8	29.2
ADPFG	21.4	13.0	21.8	34.8	27.8	26.2	28.5	11.7	09.5	20.2
ADLA	0.03	0.02	0.04	0.03	0.04	0.03	0.04	0.03	0.03	0.03