

FASEB ABSTRACT NO. 4415

PLATELET RESPONSE IN HYPERCHOLESTEROLEMIC BLOOD TO THROMBIN-INDUCED AGGREGATION. D.J. SAXON AND C.E. HAMMOND. MOREHEAD STATE UNIVERSITY, MOREHEAD, KY. 40351.

THE OBJECTIVE OF THE STUDY WAS TO DETERMINE IF HYPERCHOLESTEROLEMIC PLATELETS ARE MORE RESPONSIVE THAN NORMOCHOLESTEROLEMIC PLATELETS TO THROMBIN-INDUCED AGGREGATION IN WHOLE BLOOD. BLOOD SAMPLES FROM MALE RATS ON AN 8 WEEK DIET ENRICHED WITH 5% CHOLESTEROL + 1% SODIUM TAUROCHOLATE WERE COMPARED TO THOSE FROM NORMAL DIET RATS. SIGNIFICANT DIFFERENCES ($P < 0.05$) IN PLASMA WERE ELEVATION OF TOTAL CHOLESTEROL AND LDL, AND A DECREASE IN HDL; WHILE TRIGLYCERIDE WAS UNCHANGED BY THE ENRICHED DIET. PLATELET COUNTS WERE NOT SIGNIFICANTLY DIFFERENT. AGGREGATION WAS MEASURED IN 3.2% CITRATED BLOOD (1:9) WITH TYRODE'S Ca^{++} -FREE BUFFER (1:1) AT pH 7.4 USING IMPEDANCE AGGREGOMETRY (CHRONOLOG). HYPERCHOLESTEROLEMIC PLATELETS HAD A HIGHER RELATIVE FREQUENCY (70%) OF RESPONSE TO 0.10 UNITS OF THROMBIN COMPARED TO NORMOCHOLESTEROLEMIC PLATELETS (20%). AGGREGATION INDUCED BY 0.20 UNITS THROMBIN INCREASED THE RESPONSE BY HYPERCHOLESTEROLEMIC PLATELETS TO 86%. HYPERCHOLESTEROLEMIC PLATELETS HAD A SIGNIFICANTLY GREATER MAXIMUM IMPEDANCE INCREASE IN RESPONSE TO 0.15 UNITS OF THROMBIN AND AGGREGATED AT A SIGNIFICANTLY FASTER RATE THAN NORMOCHOLESTEROLEMIC PLATELETS. THUS HYPERCHOLESTEROLEMIA APPEARS TO HAVE A ROLE IN INCREASING PLATELET RESPONSE TO THROMBIN-INDUCED AGGREGATION AS MEASURED IN WHOLE BLOOD USING IMPEDANCE AGGREGOMETRY.

INTRODUCTION

PLATELETS RESPOND TO A VARIETY OF AGONISTS INCLUDING THROMBIN. AGGREGATION, OR PLATELET STICKING TO PLATELET, REQUIRES THE PRESENCE OF CALCIUM AND THE EXPRESSION OF FIBRINOGEN RECEPTORS ON THE PLATELET SURFACE. THIS EXPRESSION OF THE FIBRINOGEN RECEPTOR IS ASSOCIATED WITH CHANGES IN THE CONFORMATION OF GLYCOPROTEINS IIB-IIIa.

WHEN THROMBIN BINDS TO THE THROMBIN RECEPTOR AND GLYCOPROTEIN IIB-IIIa COMPLEX OF THE FIBRINOGEN RECEPTOR IS ACTIVATED AGGREGATION IS FACILITATED THROUGH LINKAGES WITH FIBRINOGEN. PLATELET AGGREGATES CAN FORM AT THE SITE OF A VESSEL WALL INJURY AND MAY HAVE A ROLE IN INITIATING THE COAGULATION PROCESS. PLATELET AGGREGATES MAY ALSO HAVE A ROLE IN ATHEROGENESIS.

HYPERCHOLESTEROLEMIA (ELEVATED LDL-CHOLESTEROL) APPEARS TO HAVE A ROLE IN ALTERING THE BIOCHEMISTRY AND THE FUNCTION OF BLOOD VESSEL CELLS AND PLATELETS. AGGREGATION STUDIES USING ISOLATED, WASHED, HYPERCHOLESTEROLEMIC, RAT PLATELETS (WINOCUR, ET AL) INDICATE AN INCREASED SENSITIVITY TO AGGREGATION BY THROMBIN AS MEASURED BY OPTICAL AGGREGOMETRY.

PLATELETS IN WHOLE BLOOD ARE CERTAINLY EXPOSED TO A MULTITUDE OF AGONISTS FROM A VARIETY OF CELLS AND IMPEDANCE AGGREGOMETRY PERMITS THE UTILIZATION OF WHOLE BLOOD TO MEASURE THIS PLATELET FUNCTION. THEREFORE THE OBJECTIVE OF THIS STUDY WAS TO DETERMINE IF HYPERCHOLESTEROLEMIC PLATELETS ARE MORE RESPONSIVE THAN NORMO-CHOLESTEROLEMIC PLATELETS TO THROMBIN-INDUCED AGGREGATION IN WHOLE BLOOD AS MEASURED BY IMPEDANCE AGGREGOMETRY.

METHODS

DIETARY GROUPS OF MALE SPRAGUE-DAWLEY RATS (275-299 GRAMS):

1. NORMAL DIET - PURINA 5001 LABORATORY RODENT CHOW FOR 8 WEEKS.
2. HIGH CHOLESTEROL DIET - PURINA 5001 LABORATORY RODENT CHOW CONTAINING 5% CHOLESTEROL + 1% SODIUM TAUROCHOLATE FOR 8 WEEKS.

BLOOD SAMPLES:

TAIL ARTERIAL BLOOD + 3.2% SODIUM CITRATE (9:1 RATIO)

DETERMINATION OF PLASMA LIPID LEVELS

1. TOTAL CHOLESTEROL: MODIFICATION OF ALLAIN ET AL PROCEDURE (1974)
SIGMA DIAGNOSTICS PROCEDURE NO. 352
2. TRIGLYCERIDES: MODIFICATION OF MCGOWAN ET AL PROCEDURE (1983)
SIGMA DIAGNOSTICS PROCEDURE NO. 339
3. HDL-CHOLESTEROL: WARNER ET AL PROCEDURE (1982)
SIGMA DIAGNOSTICS PROCEDURE NO. 352-3
4. LDL-CHOLESTEROL: CALCULATION BASED ON FRIEDEWALD ET AL (1972)

PLATELET COUNTS:

PLATELET COUNTS OF PRP WERE DETERMINED WITH A COULTER Z_F COUNTER.

MEASUREMENT OF THROMBIN-INDUCED PLATELET AGGRETATION IN WHOLE BLOOD

1. BLOOD SAMPLES WERE PREPARED IN A 1:1 RATIO (0.5 ML + 0.5 ML) OF CITRATED BLOOD AND CALCIUM - FREE TYRODE SOLUTION CONTAINING ALBUMIN, pH 7.4.
2. THROMBIN LEVELS USED WERE 0.05 TO 0.30 UNITS PER ML OF FINAL VOLUME
3. MEASUREMENT OF PLATELET AGGREGATION WAS PERFORMED IN A CHRONO-LOG WHOLE BLOOD AGGREGOMETER.

ANALYSIS OF DATA:

1. RELATIVE FREQUENCIES OF THE CONTROL GROUP AND EXPERIMENTAL GROUP RESPONSES TO THROMBIN WERE COMPARED.
2. MAXIMAL IMPEDANCE & MAXIMAL RATE OF IMPEDANCE CHANGE OF THE CONTROL GROUP AND EXPERIMENTAL GROUP RESPONSES TO THROMBIN WERE COMPARED.

RESULTS

PLASMA LIPID LEVELS, EXCEPT TRIGLYCERIDES, WERE SIGNIFICANTLY ALTERED BY THE HIGH CHOLESTEROL DIET. TOTAL CHOLESTEROL AND LDL-CHOLESTEROL WERE ELEVATED, WHILE HDL-CHOLESTEROL DECREASED (TABLE 1).

THERE WAS NO SIGNIFICANT DIFFERENCE IN PLATELET COUNTS OF RATS RECEIVING THE NORMAL DIET AND HIGH CHOLESTEROL DIET (TABLE 2).

THE MINIMUM AMOUNT OF THROMBIN WHICH INDUCED AGGREGATION IN EITHER GROUP WAS 0.15 UNITS (FIGURES 1 AND 2). TABLE 3 ILLUSTRATES THAT THE HIGH CHOLESTEROL DIET GROUP HAD A GREATER RELATIVE FREQUENCY OF RESPONSE (70%) TO THE 0.15 UNITS OF THROMBIN COMPARED TO THE RELATIVE FREQUENCY OF RESPONSE (20%) BY THE NORMAL DIET GROUP. THE RELATIVE FREQUENCY OF THROMBIN-INDUCED PLATELET AGGREGATION INCREASED IN THE HIGH CHOLESTEROL GROUP WHEN THE THROMBIN WAS INCREASED TO 0.20 UNITS (TABLE 4).

FIGURES 1 AND 2 ILLUSTRATE TYPICAL THROMBIN-INDUCED AGGREGATION RESPONSES IN BLOOD TO 0.10, 0.15, 0.20 AND 0.25 UNITS OF THROMBIN BY RATS FROM EACH DIET GROUP. AGGREGATION RESPONSES WERE MEASURED BY THE MAXIMAL CHANGE IN IMPEDENCE (MAXIMUM OHMS) AND BY MAXIMAL RATE OF IMPEDENCE CHANGE (DELTA OHMS PER SECOND).

WHEN 0.15 UNITS OF THROMBIN WAS TESTED ON BOTH GROUPS, 80% OF THE NORMAL DIET GROUP'S BLOOD SAMPLES HAD NO RESPONSE. THE OTHER NORMAL DIET SAMPLES HAD ONLY SLIGHT RESPONSES. IN THE HIGH CHOLESTEROL DIET GROUP 30% DEMONSTRATED NO RESPONSE, BUT THE REMAINING 70% OF THE HIGH CHOLESTEROL DIET GROUP DEMONSTRATED A SIGNIFICANTLY GREATER RESPONSE TO THE 0.15 UNITS. THE HIGH CHOLESTEROL GROUP DEMONSTRATED A SIGNIFICANTLY GREATER RESPONSE TO THROMBIN THAN ANY OF THOSE ELICITED BY AN EQUAL AMOUNT OF THROMBIN ADDED TO THE NORMAL GROUP'S BLOOD. (FIGURES 1 & 2 AND TABLE 5).

TABLE 1: PLASMA LIPID LEVELS OF RATS FED FOR 8 WEEKS ON A NORMAL DIET OR A HIGH CHOLESTEROL DIET CONTAINING 5% CHOLESTEROL AND 1% SODIUM TAUROCHOLATE

Plasma Lipid	N Diet Rats		HC Diet Rats		t-test
	<u>n</u>	<u>mg%</u>	<u>n</u>	<u>mg%</u>	
Tc	10	10.5 +/- 13.8	7	199.2 +/- 24.8	S
LDL	10	6.2 +/- 10.0	7	152.0 +/- 30.4	S
HDL	10	58.2 +/- 8.2	7	38.2 +/- 8.2	S
TG	10	48.9 +/- 15.9	7	44.2 +/- 16.1	NS

mg% as mean, +/- standard deviation.

n = number of animals in diet group.

N = normal diet, HC = high cholesterol diet.

Tc = total cholesterol, LDL = LDL cholesterol.

HDL = HDL cholesterol, TG = triglycerides.

S = significant at $p < 0.05$

NS = not significant at $p < 0.05$

TABLE 2: PLATELET COUNTS OF RATS FED FOR 8 WEEKS ON A NORMAL DIET OR A HIGH CHOLESTEROL DIET CONTAINING 5% CHOLESTEROL AND 1% SODIUM TAUROCHOLATE

<u>Diet</u>	<u>n</u>	<u>Platelet Counts in Millions</u> <u>Per mL of Whole Blood</u>	<u>t-test</u>
N	7	107.705 +/- 48.124	NS
HC	10	101.090 +/- 25.855	

N = normal diet, HC = high cholesterol diet.

n = number in diet group.

Platelet counts as mean +/- standard deviation.

NS = not significant at $p < 0.05$.

TABLE 3: RELATIVE FREQUENCIES OF PLATELET AGGREGATION FOLLOWING ADDITION OF 0.15 UNITS OF THROMBIN TO BLOOD FROM RATS FED FOR 8 WEEKS ON A NORMAL DIET OR A HIGH CHOLESTEROL DIET CONTAINING 5% CHOLESTEROL AND 1% SODIUM TAUROCHOLATE

<u>Diet Group</u>	<u>n</u>	<u>Frequency of Aggregation</u>	<u>Percentage</u>
N	10	2	20%
HC	10	7	70%

N = normal diet, HC = high cholesterol diet.
n = number in diet group.

TABLE 4: RELATIVE FREQUENCIES OF PLATELET AGGREGATION FOLLOWING ADDITION OF 0.20 UNITS OF THROMBIN TO BLOOD FROM RATS FED FOR 8 WEEKS ON A NORMAL DIET OR HIGH CHOLESTEROL DIET CONTAINING 5% CHOLESTEROL AND 1% SODIUM TAUROCHOLATE

<u>Diet Group</u>	<u>n</u>	<u>Frequency of Aggregation</u>	<u>Percentage</u>
N	10	2	20%
HC	7	6	85.7%

N = normal diet, HC = high cholesterol diet.
n = number in diet group.

TABLE 5: THROMBIN-INDUCED AGGREGATION FOLLOWING ADDITION OF 0.15 UNITS OF THROMBIN TO BLOOD FROM RATS FED FOR 8 WEEKS ON A NORMAL OR A HIGH CHOLESTEROL DIET CONTAINING 5% CHOLESTEROL AND 1% SODIUM TAUROCHOLATE

<u>Diet Group</u>	<u>n</u>	<u>Delta Ohms/Sec</u>	<u>Maximum Delta Ohms</u>
N	8	0.002 +/- 0.004	0.125 +/- 0.2673
HC	10	0.042 +/- 0.055	2.875 +/- 4.389
t-test		p < 0.025	p < 0.05

N = normal diet, HC = high cholesterol diet.
 Maximum Delta Ohms/Sec as means +/- standard deviation.
 Maximum Delta Ohms as means +/- standard deviation.

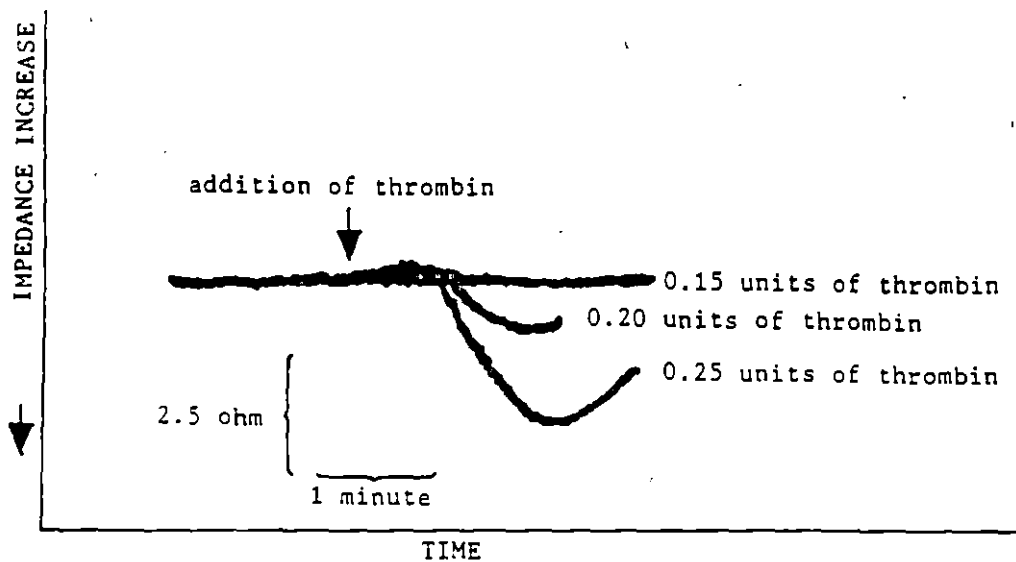


Figure 1: Aggregation responses of 1 mL blood (0.5 mL citrated blood:0.5 mL Tyrode calcium-free buffer, pH 7.4) from the normal diet group of animals to 0.15, 0.20, and 0.25 units of thrombin

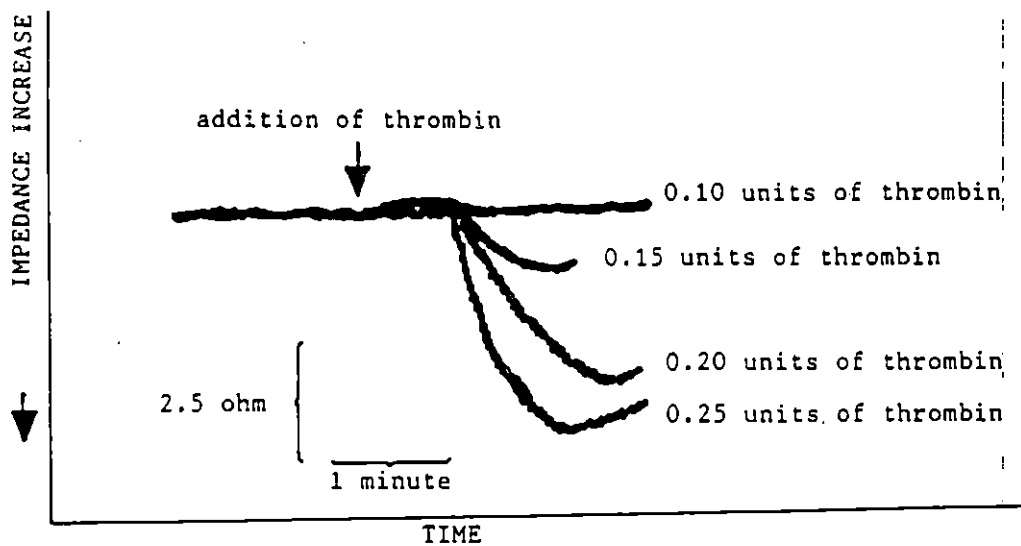


Figure 2: Aggregation responses of 1 mL blood (0.5 mL citrated blood:0.5 mL Tyrode calcium-free buffer, pH 7.4) from the high cholesterol diet group of animals to 0.10, 0.15, 0.20, and 0.25 units of thrombin

CONCLUSIONS

1. HYPERCHOLESTEROLEMIA DOES APPEAR TO HAVE A ROLE IN ALTERING PLATELET RESPONSE TO THROMBIN-INDUCED AGGREGATION AS MEASURED IN WHOLE BLOOD USING IMPEDANCE AGGREGOMETRY.

2. THE ALTERATIONS IN PLATELET RESPONSE TO THROMBIN INCLUDE:
 - A. A SIGNIFICANTLY GREATER MAXIMUM AGGREGATION RESPONSE BY HYPERCHOLESTEROLEMIC PLATELETS,
 - B. AN INCREASE IN THE RATE OF AGGREGATION BY THESE HYPERCHOLESTEROLEMIC PLATELETS AND
 - C. INDUCTION OF AGGREGATION BY HYPERCHOLESTEROLEMIC PLATELETS REQUIRES LESS THROMBIN THAN NORMOCHOLESTEROLEMIC PLATELETS.