Effect of Age and Restraint on Postprandial Lipemia Clearance in Dogs.* (27993)

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It has been observed that patients with ischemic heart disease exhibit more marked and more prolonged postprandial hyperlipemia than normal persons(1-4). It is, therefore, desirable to determine the factors which influence the duration of lipemia following a fat meal. Both stress phenomena and age have been reported to influence lipid metabolism generally(5). Stress phenomena may cause increased serum NEFA (non-esterified fatty acids), lipemia, phospholipid and cholesterol levels. We have observed that an atherogenic regimen fed to "middle aged" dogs may lead to myocardial infarction while it does not do so in young dogs(6). An investigation was accordingly undertaken to study the effect of age and stress on postprandial lipemia clearance in dogs. It was observed that, except for the very old dogs, following a fat meal rate of lipemia clearance was reduced with advancing age. However, contrary to expectation, dogs which were exposed to restraint seemed to develop lower levels of lipemia and NEFA concentration did not increase.

Procedure. Male dogs were obtained from a commercial supplier and were in good health as far as could be determined by gross examination. The age of each animal was established either from the records of the original owner or by 3 experienced individuals. Disagreement occurred only when ascertaining the age of animals over 9 years of age.

After 2 days of acclimatization dogs were subjected to a 16 hour overnight fast, with free access to water. The bladder was emptied of its urine contents by catheterization. A sample of blood was drawn. One hundred grams of lean ground meat was combined with lard (1 g per lb body weight) and fed to each animal. Specimens of blood were drawn at 2, 4 and 6 hours. The bladder was emptied at 3 and 6 hours and the urine combined. During this time the animal remained in his cage and had free access to water. After collections were complete the dog was fed a basal commercial dog meal. The next day the experiment was repeated except that the animal was restrained in a modified Pavlov rig provided with a stock to hold the head.† This permitted movement of the head and the limbs, but only slight movement of the body. This procedure results in a large increase in excretion of urinary corticoids.† The dogs respond with varied patterns of behavior to gain release. Some struggle slightly at intermittent periods, others more frequently and more vigorously, and some quite vigorously with ¼ or more of the time spent in the rig given to this activity.

Urinary corticoids were determined by the procedure of Silber and Porter(7). NEFA were determined by the procedure of Dole(8) with the exception that Nile blue was used in the titration. Urinary catecholamines were determined by the procedure given by Straus and Wurm(9). Lipemia, due to the presence of triglyceride-rich chylomicrons, was determined by the turbidimetric method of Osmon *et al.*(10). The optical density reading on the Beckman spectrophotometer $\times 100$ was taken as the arbitrary units of lipemia.

Results. Thirty-seven dogs were used in the experiment. Ten were less than 1 year of age (Group 1), 8 were 1 to 3 years old (Group 2), 10 were 4 to 8 years old (Group 3) and 9 were over 9 years old (Group 4).

Control period. The results with lipemia clearance are shown in Table I and Fig. 1. In Groups 1 to 3 peak lipemia was observed 4 hours after ingestion of the test meal. The values for Group 2 were greater than those of Group 1 after 2 hours and 4 hours (increments of 280U and 576U as compared to 115U and 408U). The 6 hour values were equivalent (335U and 377U). In turn, each of the values for Group 3 was greater than that of Group 2 (increments of 533U, 1047U and 819U). However, Group 4 dogs exhibited

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† Sobel, H., Mondon, C. E., Harvey, H., unpublished observations.

TABLE I. Postprandial Lipemia Clearance in Dogs.

Group	Age, yr	Fasting	Control period 2 hr	Control period 4 hr	Control period 6 hr	Restraint period 2 hr	Restraint period 4 hr	Restraint period 6 hr
1	<1	115[1] ± 21[2]	115[3] ± 21	408 ± 28	377 ± 72	285 ± 63†$^{+}$	372 ± 68	193 ± 52†$^{-}$
2	1–3	105 ± 11	280 ± 42*$^{+}$	576 ± 82*$^{+}$	335 ± 63	246 ± 35	416 ± 66	335 ± 96
3	4–8	144 ± 18	533 ± 80*$^{+}$	1047 ±121*$^{+}$	819 ± 52*$^{+}$	277 ± 94†$^{-}$	506 ± 96†$^{-}$	553 ±112†$^{-}$
4	>9	157 ± 20	254 ± 66*$^{-}$	378 ± 47*$^{-}$	669 ± 81	248 ± 64	549 ±101	295 ±137†$^{-}$

[1] Arbitrary units as defined in *Procedure*. [2] Standard error. [3] Increment above fasting level.
* Statistically significant difference as compared to value above ($^{+}$ more, $^{-}$ less).
† Statistically significant difference from corresponding control period ($^{+}$ more, $^{-}$ less).

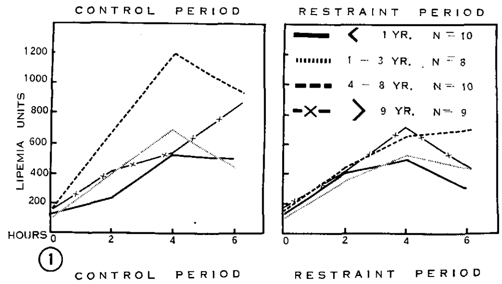
TABLE II. Postprandial Serum NEFA Values.

Group	Age, yr	Fasting	Control period 2 hr	Control period 4 hr	Control period 6 hr	Restraint period 2 hr	Restraint period 4 hr	Restraint period 6 hr
1	<1	370[1] ± 65	466 ± 42	488 ± 63	593 ± 61*	508 ± 21	517 ± 36	441 ± 23†
2	1–3	372 ± 19	412 ± 14	558 ± 67*	437 ± 26	420 ± 32	454 ± 37	414 ± 44
3	4–8	353 ± 16	449 ± 32*	470 ± 47*	483 ± 33*	397 ± 20	442 ± 33	476 ± 34
4	>9	425 ± 28	478 ± 33	548 ± 46*	551 ± 32*	556 ± 55	562 ± 48	523 ± 28

* Statistically significant increase as compared to fasting value. [1] μM/l.
† Statistically significant decrease from corresponding control period.

quite another pattern with values below those of Group 3 and highest value observed after 6 hours (increments of 254U, 378U and 669U).

The corresponding NEFA values for each period were similar to those observed by Ballard *et al.*(11) following a fatty meal (Table II and Fig. 2). In the postprandial state the



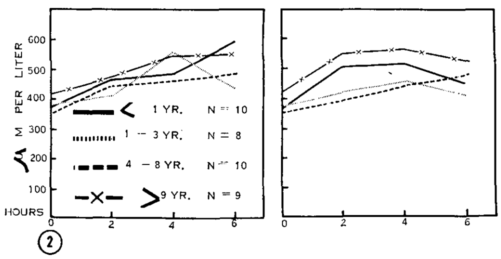


FIG. 1. Avg lipemia for each age group at 2 hr intervals following test meal containing fat. N = No. in each group.

FIG. 2. Avg NEFA values for each age group at 2 hr intervals following test meal containing fat. N = No. in each group.

values were greater than those of the fasting state; in a number of instances these differences were statistically significant. The greatest values were observed following the 4 or 6 hour periods with increases of 30% to 60% above the fasting state. There was no apparent trend with reference to age.

Effect of restraint. During 6 hours of restraint the dogs responded as indicated above. It is of interest that the number of dogs in Groups 1-4 whose attempts to gain release might be classified as vigorous were 2, 5, 7 and 3 respectively.

As previously indicated, restraint proved to be a powerful stressor. Average increase in urinary corticoid excretion for each group was 3.5-4.0 times greater than that of the 6 hour basal collection period (Table III). Urinary catecholamines, not previously determined during this operation, were increased 4.0-4.6 times. Average increase in urine volume exceeded 60% for all groups. There was a tendency for the older animals to exhibit greater absolute increments. For Groups 3 and 4 the increment in corticoid excretion during restraint was 529 μg and 606 μg respectively as compared to 452 μg and 431 μg for Groups 1 and 2. Similarly for catecholamine excretion the increments were 10.8 μg and 11.7 μg as compared to 9.0 μg and 8.1 μg. Increment in urine volume was somewhat greater in the

TABLE III. Urinary Catecholamines, Corticoids and Volume in Dogs under Restraint.

Group	Age, yr	Catecholamines C	Catecholamines R	Corticoids C	Corticoids R	Urine volume C	Urine volume R
1	<1	3.4[1] ± .2	12.4* ± 1.2	134[1] ± 9	586* ± 45	43[2] ± 3	72* ± 6
2	1–3	3.3 ± .2	11.4* ± 1.5	144 ± 11	575* ± 49	37 ± 4	60 ± 30
3	4–8	3.6 ± 1.0	14.4* ± 1.6	146 ± 15	675* ± 60	51 ± 3	85* ± 8
4	>9	4.0 ± .7	15.7* ± 3.3	180 ± 117	786* ± 117	54 ± 9	87* ± 9

C = Control period. [1] μg/6 hr collection period. [2] cc/6 hr collection period.
R = Restraint " .
* Statistically significant increase as compared to control period.

older dogs during this period.

During the restraint peak lipemia levels were definitely lowered for Groups 1-3 (by 9%, 28% and 52% respectively) although only in the case of the latter were the differences statistically significant (Table I). For Group 1 after 2 hours the lipemia values were actually significantly greater (2.4 times) than during the control period; however, the average was slightly lower at 4 hours and significantly lower after 6 hours. Group 2 dogs showed a tendency for lower values at 2 hours and 4 hours. In Group 3 dogs, each of the values was markedly less than that of the corresponding control period. In Group 4 the pattern now was similar to that of the others, with the greatest value observed after 4 hours, actually an increment of 45%higher than that of the control period, and a reduction after 6 hours, markedly below that of the control period.

The NEFA values did not differ dramatically from those observed during the control period. There were relatively small increments and decrements without any definite pattern. The greatest increase (16%) was observed after 2 hours in the Group 4 dogs and greatest decrease (–26%) after 6 hours in the Group 1 dogs.

Discussion. Although the findings were not statistically significant in all cases, they suggest that postprandial lipemia clearance in dogs up to 9 years of age is progressively delayed. Delayed lipemia clearance with age has been observed frequently in man. In early studies with the chylomicron counting technic Becker and co-workers(12) found a higher peak and marked delay in return to basal levels in aged subjects. More recently Brown *et al.*(13) using I^{131}-labeled fat observed that healthy middle-aged men cleared fat more slowly than younger men.

In the case of Group 4, the time or degree of peak lipemia is not known because the observation period did not exceed 6 hours. However, the findings suggest delayed intestinal absorption as compared with Group 3. Whether this is due to reduction in pancreatic lipase, reduced intestinal motility, a manifestation of the age-associated impaired transport mechanism(14) at the intestinal level or any other mechanism is not clear.

Restraint resulted in large increase in catecholamine and corticoid excretion. From the known effects of catecholamines, ACTH, and corticoids on lipid metabolism(5), it was expected that plasma NEFA would increase, and lipemia clearance would be unaffected or inhibited following exposure to a stressor. Instead, NEFA levels were unaffected and lipemia clearance in general seemed to be accelerated as compared to the control periods in Groups 1-3. In Group 4 the peak occurred after 4 hours under restraint in contrast to 6 hours during the control period. Possibly this was due to increased intestinal motility.

How does one reconcile the findings with regard to NEFA and lipemia clearance with that which was expected? It will be recalled that some dogs made vigorous attempts to withdraw from the rig. These consituted 20%, 62%, 70% and 33% respectively of Groups 1-4. When the decrement in lipemia was obtained by measuring the areas under the clearance curves during the control and restraint periods according to an arbitrary scale, values of 33, 128, 582 and 10 respectively were found, suggesting a possible correlation.

Nikkilä and Konttinen have observed that following a meal containing milk-fat, serum triglyceride and NEFA levels were lower in a group of men who marched for 2 hours as compared to their controls who rested in bed

(15). Issekutz and Miller(16) observed that in dogs NEFA levels rapidly decreased during exercise. These findings suggest that expected effects of stress-released catecholamines and corticoids were not observed because of the increased caloric expenditure of the dogs in restraint.

Summary. A meal containing 1 g of lard per pound body weight in a meat patty was given to male mongrel dogs after a 16 hour fast. Lipemia clearance was observed in dogs of 4 age groups as follows:

Group 1, less than 1 year, Group 2, 1 to 3 years, Group 3, 4 to 8 years and Group 4, over 9 years. Peak lipemia was apparent after 4 hours in Groups 1-3 and was greater in Group 2 than in Group 1 and in Group 3 than in Group 2. In Group 4 dogs peak lipemia occurred later than in the others but was not as great as that observed in Group 3. During restraint in a modified Pavlov rig for 6 hours urinary catecholamine and corticoid excretion increased approximately 4-fold without regard to age. Contrary to expectation, lipemia clearance was accelerated and serum NEFA did not rise. Increased clearance seemed to be correlated with activity in the rig.

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Angiotensin II Degradation by Human Cohn Plasma Fractions.* (27994)

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Plasma or serum from patients with untreated primary benign hypertension(1) degrades more angiotensin II-I^{131} *in vitro* than plasma or serum from normotensive or secondary renal hypertensive subjects(2-7). Similarly, the *in vitro* biological half-life of angiotensin II is shorter in patients with primary hypertension than in normotensive subjects(8). Previous heat inactivation of the serum or plasma, before reaction with the angiotensin II-I^{131} *in vitro*, increases the amount of angiotensin II-I^{131} degraded(2,7, 9). These observations prompted investigation of the identity of the plasma fractions which enhance and inhibit angiotensin II-I^{131} degradation.

Methods. Angiotensin II-I^{131} was prepared by the Newerly(10) modification of the Pressman and Eisen method. Twelve different Cohn fractions prepared from different batches of pooled human plasma were obtained. Various concentrations of each of the

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