Effect of Atherosclerosis and Vitamin A on Glucose Tolerance in Dogs.* (32233)

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For the past several years this laboratory has been studying biochemical lesions associated with the development of atherosclerosis in purebred beagles. Since it has been reported that individuals afflicted with this disease have impaired glucose tolerances(1,2) we have examined this parameter in atherosclerotic dogs. We have also studied the glucose tolerance of atherosclerotic dogs supplemented with vitamin A because of our interest in the effect of this vitamin on the development of this vascular disease.

In this report we demonstrate that atherosclerotic dogs did not show impaired glucose tolerances but when they were supplemented with vitamin A they did exhibit an increased tolerance to glucose.

Methods. Nineteen purebred beagles (6 months old), F_2's of sibbed, F_1's were divided into 3 groups. A control group of animals, 3 males and 1 female, were maintained on an *ad libitum* diet of Purina dog chow. The atherosclerotic group, 5 males and 5 females, were given initially 40 units of ACTH gel subcutaneously every other day for 6 days (total of 3 injections) and fed daily 0.5 g of thiouracil and a special diet *ad libitum* composed of 92% Purina dog chow, 5% polyunsaturated vegetable oil, 3% cholesterol plus 3 egg yolks per 500 g food. The approximate daily intake of each dog was 500 g of food. The effectiveness of this regime in producing atherosclerotic change in segments of the beagle aorta has been reported(3). The atherosclerotic plus vitamin A group, 4 males and 4 females, was treated in the same manner as the atherosclerotic group, plus the daily oral administration of 5000 I.U. of water miscible vitamin A palmitate. The experimental period lasted one year.

Initial glucose tolerances were performed prior to the beginning of the experimental period (6 months old) and again at 3, 6 and 12 month intervals.

Intravenous glucose tolerance tests were carried out according to the methods of Camerini-Davalos *et al*(4) and Silverstone *et al*(5). Five-tenths of a gram of glucose per kilogram body weight (0.5 g/kg) of a 50% glucose solution was injected intravenously in the course of 4 minutes. Blood sugar levels were determined by Nelson's adaptation of the method of Somogyi(6).

The blood sugar diminution rate (k-value) for each dog was calculated according to the method of Lundbaek(7).

Since thiouracil and perhaps vitamin A(8) influences the condition of the thyroid gland, serum protein-bound iodine was determined on all dogs initially and at termination of the experimental period according to the method of Barker as modified by Foss *et al*(9).

Results. The average blood glucose levels and blood sugar diminution rate (k-value) during the tolerance test for the 3 groups of dogs are presented in Tables I and II, respectively. It may be noted from the control group that there was no significant change in the glucose tolerance of the dogs during the 12 months of the experimental period. Likewise the atherosclerotic group showed no significant change in glucose tolerance during the atherogenic stress period. The atherosclerotic group plus vitamin A did, however, demonstrate an increase in glucose utilization when compared with initial glucose tolerances. This effect is best demonstrated when the initial k-values (rate of glucose diminution) are compared with similar values during the experimental period (Table II). Such a comparison reveals that the initial k-values for the atherosclerotic plus vitamin A group were significantly lower than the k-values found for the 3, 6 and 12 month periods of the experiment.

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TABLE I. Mean Blood Glucose Levels in I.V. Glucose Tolerance Tests (mg/100 ml).

	Fasting	10 min	20 min	40 min	60 min	100 min
Control group						
Initial	70 ± 2*	140 ± 5	120 ± 2	100 ± 1	88 ± 5	70 ± 1
6th month	68 ± 3	136 ± 17	112 ± 14	94 ± 12	79 ± 6	70 ± 5
12th "	65 ± 10	155 ± 18	124 ± 18	91 ± 28	72 ± 11	68 ± 7
Atherosclerotic group						
Initial	58 ± 8	141 ± 9	116 ± 36	98 ± 3	90 ± 16	66 ± 1
3rd month	73 ± 8	158 ± 16	121 ± 21	98 ± 19	81 ± 12	83 ± 13
6th "	69 ± 10	147 ± 26	112 ± 16	89 ± 18	81 ± 14	77 ± 11
12th "	68 ± 9.5	143 ± 19	114 ± 11	80 ± 5	73 ± 5.5	72 ± 13
Atherosclerotic + vitamin A group						
Initial	73 ± 3	145 ± 1	—	101 ± 7	86 ± 7	77 ± 3
3rd month	66 ± 5	161 ± 11	101 ± 22	79 ± 11	78 ± 11	76 ± 9
6th "	75 ± 11	173 ± 24	129 ± 15	88 ± 8	78 ± 3	75 ± 7
12th "	61 ± 12	160 ± 31	111 ± 22	73 ± 18	68 ± 14	65 ± 13

* Standard deviation.

Average serum vitamin A levels of the dogs receiving vitamin A for 12 months was 1050 ± 20 g/100 ml, while the average levels for the control and atherosclerotic group were 213 ± 50 g/100 ml and 230 ± 30 g/100 ml, respectively. These data indicate that the vitamin A administered was absorbed.

The average serum protein-bound iodine values for the 3 groups of dogs at the beginning and termination of the experimental period were not significantly different (*e.g.*, average initial level of control group 3.8 ± 0.3 μg/100 ml). Therefore using serum protein-bound iodine levels as an index of thyroid function it would appear that neither thiouracil nor vitamin A significantly altered thyroid function.

Discussion. The intravenous glucose tolerance method was selected for this study because of technical conveniences and because of certain advantages this method has over oral administration in measuring glucose tolerance(7). The interpretation of results must be considered, however, in light of the technique used. There are conflicting results on glucose utilization in atherosclerotic subjects where oral(1,2,10,11) and intravenous(12,13) methods have been used. These differences are understandable since glucose utilization after oral administration depends not only on homeostatic factors affected by elevated blood sugar levels but it is also influenced by such gastrointestinal factors as absorption rate and hormonal release. Therefore, the results in this study should be compared with similar studies using intravenous techniques in examining glucose utilization in subjects with atherosclerosis. Our results are in agreement with the findings of Ryan(12) and Rifkind (13) who showed that glucose utilization in atherosclerosis is not impaired following I.V. glucose administration.

TABLE II. Mean k-Values of Glucose Tolerance Tests in the Purebred Beagles. (Percentage fall of blood glucose per minute.)

	Control group	Atherosclerotic group	Atherosclerotic + vitamin A group
Initial	1.51 ± .4*	1.71 ± .6	1.31 ± .5
3rd month	—	2.02 ± .7	2.82 ± .8↑
6th "	1.31 ± .3	1.68 ± .3	2.53 ± .7↑
12th "	2.09 ± .5	1.74 ± .5	3.29 ± 1.3↑

↑ Indicates significant increased glucose utilization as determined by "t" values. $p < .01$.
* Standard deviation.

It should be pointed out, however, that the above findings pertain only to experimental dogs fed an atherogenic diet and do not disprove the possibility that a basic metabolic defect in glucose tolerance may be related to atherosclerosis in man.

The second significant finding of this study was the increased glucose tolerance in atherosclerotic dogs supplemented with vitamin A. The significant increase in k-values over initial values for this group of dogs (Table II) during the 3rd, 6th and 12th months of the experiment supports this conclusion. The mechanism whereby vitamin A brings about this effect of enhanced glucose utilization remains to be elucidated. In light of previous

observations on the effect of vitamin A on cell membranes(14) it is interesting to speculate that this effect of vitamin A on increased glucose utilization may be the result of increased permeability of cell membranes to glucose.

Summary. Intravenous glucose tolerance studies showed no impairment of glucose utilization in atherosclerotic dogs. It was observed, however, that administration of vitamin A to atherosclerotic dogs did produce a significant increase in glucose utilization.

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Effect of Aldosterone on Transmembrane Potentials of Frog Skeletal Muscle.* (32234)

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The importance of aldosterone in sodium and potassium regulation by the kidney of mammals is well established(1). More attention has been given to its renal than to its extra-renal activity. Its role in sodium transport is most clearly delineated by studies on amphibian skin and bladder(2,3). Since the effect of aldosterone is on sodium transport, it is not unexpected that administration of this substance to mice produced a decrease of intra-cellular sodium and an increase of intra-cellular potassium(4). Administration of the hormone to rats raised the resting membrane potentials of muscles which had been previously depressed by adrenalectomy (5). In experiments reported here aldosterone is shown to have an acute effect on resting potentials of amphibian skeletal muscle *in vitro*.

Methods. Resting potentials were examined in sartorius muscles isolated from small (2-3 inch) *Rana pipiens* which had been confined for 24 hours in cages containing a moderate amount of distilled water at 4°C. Potentials were determined at room temperature (25°C) with glass microelectrodes filled with 2.7 M KCl and connected to the input probe of a negative-capacity electrometer amplifier (Medistor Instr. Co., Model A-35) through a saturated KCl solution and calomel half-cell. The reference lead in the solution bathing the muscles was an agar-Ringer bridge connected to the amplifier through a second saturated KCl solution and calomel half-cell. Microelectrode resistances were between 8 and 15 megohms. Output of the amplifier was monitored on an oscilloscope and recorded on a servo-recorder (Heath Co., Model EUW-

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