Age and Genetic Effects on pH Changes in Adipose Tissue

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Age and genetic (animal breed) effects on changes in adipose tissue (*panniculus adiposus*) pH and hydrogen ion concentration were studied. Initial (0-hour) pH were shown to increase ($P < 0.01$) with age in both the outer and inner layers of the *panniculus adiposus*. The reverse trend with age was noted for 4-hour and ultimate (24-hour) pH values. The genetic effect on pH changes in adipose tissues was demonstrated, particularly in older animals. Increasing animal age also resulted in greater pH and hydrogen ion concentration changes between initial (0-hour) and ultimate (24-hour) measurements in both layers of the *panniculus adiposus*. Only in the older (225-day) animals did animal breed affect these changes.

Aging is the result of processes taking place at subcellular and molecular levels (Sorokin, 1964). Tissue pH changes have been accepted as an indication of the underlying, concomitant physical and chemical changes (Lawrie *et al.*, 1958; Wismer-Pedersen and Briskey, 1961). In an earlier investigation (Sink, 1966), the two layers of the *panniculus adiposus* of *Sus domesticus* were observed to differ significantly in the rate and extent of pH changes. The present study was conducted to determine the age and genetic effects on these pH changes that occur in adipose tissue.

METHODS AND MATERIALS

Thirty castrated male animals (*Sus domesticus*) representing two genetically different breeds (Hampshire and Chester White) were used in this study. Chester Whites tend to be more obese, as measured by *panniculus adiposus* thickness, than Hampshires. After the animals were weaned, equal numbers of each breed were randomly assigned to each of three chronological age groups (125, 175, 225 days). These animals were reared on the same diet and in a controlled temperature (15° C.) environment.

As the experimental animals reached the desired age end point, they were sacrificed in the usual manner. Samples of the *panniculus adiposus* were excised from the lumbar region of the right side of the carcass immediately after exsanguination (0-hour) and at 0.5, 1, 2, 3, 4, and 24 hours post-mortem. The pH values of the outer and inner layers of this adipose tissue were measured with a Beckman Zeromatic pH meter equipped with a combination probe-electrode. The pH is related to the hydrogen ion concentration $[H^+]$ by the following expression

$$pH = -\log [H^+]$$

Consequently, in these studies, the pH values as read by the meter are converted to hydrogen ion concentrations.

The *F*-test was used to detect any differences among the means of the various groups (Snedecor, 1956). When interactions were significant ($P < 0.05$), the main effects were retested by the interaction term. Between-mean differences from group to group were tested by the multiple range method of Duncan (1955).

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RESULTS

Outer Layer. Table I summarizes the effect of age and breed on the pH changes in the outer layer of the *panniculus adiposus*. As the data indicate, there were highly significant ($P < 0.01$) age effects on the initial, 4-hour, and ultimate but not on the 0.5-, 1-, 2-, or 3-hour values. Initial pH was observed to increase with age, while just the reverse was noted for 4-hour and ultimate pH. Age also affected the extent of the 24-hour pH changes, and greater changes were observed as animal age increased. However, when pH was considered on the basis of the change in hydrogen ion concentration, no significant ($P > 0.05$) age effect was noted. Interfering with the expected age effect was an important age-times-breed interaction as a result of the inconsistent breed differences from age to age. Generally, there were no breed effects on tissue pH, either within each age group or when summed over all ages. However, Hampshire animals did exhibit significantly higher 2-hour pH values at 175 days, while in Chester Whites, there was a higher ultimate pH at 225 days. A greater $\Delta[H^+]$ between initial and ultimate values was noted in the Hampshire than in the Chester White animals at 225 days of age.

Inner Layer. The effect of age and breed on the pH changes in the inner layer of the *panniculus adiposus* is also illustrated in Table I. Highly significant age effects on initial, 4-hour, and ultimate pH values were observed. Initial pH was observed to increase from 6.95 in the younger (125-day old) to 7.25 in the older (225-day old) animals. A decrease with age was found in 4-hour and ultimate pH values. Both ΔpH and $\Delta[H^+]$ were greatly ($P < 0.01$) influenced by age. Older animals showed much greater changes than did the intermediate (175-day old) or younger animals. Tissue 0.5-hour pH was highly ($P < 0.01$) affected by animal breed when these values were summed over all ages. However, this may be attributed to the highly important breed effect on older animals. In both instances, Hampshires had higher values than Chester Whites. Hampshires also showed greater 1- and 2-hour values at 225 days and greater 1-hour values when pooled over all ages. At 225 days, the ultimate pH of Chester Whites was 5.72 and that for Hampshires 5.56.

Pooled Analysis. Table II is a summary of the pooled data analysis for the effect of age, breed, and layer on adipose tissue pH and on changes in the pH and hydrogen ion concentration. Highly significant increases in initial

Table I. Effect of Age and Breed on the pH and Hydrogen Ion Concentration in the Outer and Inner Layers of the *Panniculus Adiposus* (*Sus domesticus*)

Source of Variation	Tissue pH, Hour Post-Mortem: 0	0.5	1	2	3	4	24	Difference (0 to 24 Hours): ΔpH	$\Delta[H^+]$ Moles $\times 10^{-7}$
Outer Layer									
125 days									
Hampshire	7.09	6.81	6.67	6.56	6.51	6.51	6.23	0.86	5.37
Chester White	7.15	6.85	6.70	6.61	6.55	6.45	6.28	0.87	5.28
	0.06	0.04	0.03	0.05	0.04	0.06	0.05	0.01	0.09
175 days									
Hampshire	7.20	6.93	6.84	6.77	6.63	6.54	5.95	1.25	11.48
Chester White	7.28	6.81	6.75	6.49	6.42	6.32	5.85	1.43	14.16
	0.08	0.12	0.09	0.28[a]	0.21	0.22	0.10	0.18	2.68
225 days									
Hampshire	7.47	6.95	6.76	6.53	6.34	6.10	5.67	1.80	21.24
Chester White	7.45	6.76	6.65	6.50	6.21	6.09	5.82	1.63	14.95
	0.02	0.19	0.11	0.03	0.13	0.01	0.15[a]	0.17	6.29[b]
All breeds	[b]					[b]	[b]	[b]	
125 days	7.12	6.83	6.69	6.59	6.53	6.48	6.26	0.87	5.33
175 days	7.24	6.87	6.80	6.63	6.53	6.43	5.90	1.34	12.82
225 days	7.46	6.86	6.71	6.52	6.28	6.09	5.75	1.72	18.09
Difference[a]	0.09	...	...	...	...	0.21	0.16	0.17	...
All ages									
Hampshire	7.25	6.90	6.76	6.62	6.49	6.38	5.95	1.30	12.70
Chester White	7.29	6.81	6.70	6.53	6.39	6.28	5.98	1.31	11.46
	0.04	0.09	0.06	0.09	0.10	0.10	0.03	0.01	1.24
Inner Layer									
125 days									
Hampshire	6.93	6.70	6.53	6.46	6.42	6.42	6.13	0.80	6.96
Chester White	6.96	6.66	6.50	6.46	6.36	6.29	6.10	0.86	7.70
	0.03	0.04	0.03	0.00	0.06	0.13	0.03	0.06	0.74
175 days									
Hampshire	7.02	6.80	6.74	6.68	6.56	6.45	5.83	1.19	15.93
Chester White	7.06	6.54	6.54	6.37	6.30	6.20	5.78	1.28	16.18
	0.04	0.26	0.20[a]	0.31[a]	0.26	0.25	0.05	0.11	0.25
225 days									
Hampshire	7.26	6.83	6.70	6.48	6.26	5.94	5.56	1.70	27.14
Chester White	7.23	6.50	6.48	6.37	6.16	5.92	5.72	1.51	18.50
	0.03	0.3 [a]	0.22	0.11	0.10	0.02	0.16[b]	0.19	8.64[b]
All breeds	[b]					[b]	[b]	[b]	[b]
125 days	6.95	6.68	6.52	6.46	6.39	6.36	6.12	0.83	7.33
175 days	7.04	6.67	6.64	6.53	6.43	6.33	5.81	1.24	16.06
225 days	7.25	6.67	6.59	6.43	6.21	5.93	5.64	1.61	22.82
Difference[a]	0.12	...	...	...	...	0.19	0.16	0.19	5.08
All ages									
Hampshire	7.07	6.78	6.66	6.54	6.41	6.27	5.84	1.23	16.48
Chester White	7.08	6.57	6.51	6.40	6.27	6.14	5.87	1.22	14.13
	0.01	0.21[b]	0.15[a]	0.14	0.14	0.13	0.03	0.01	2.55

[a] $P < 0.05$.
[b] $P < 0.01$.

Table II. Summary of the Pooled Data Analysis for the Effect of Age, Breed, and Layer on the pH and Hydrogen Ion Concentration of the *Panniculus Adiposus* (*Sus Domesticus*)

Source of Variation	Tissue pH, Hour Post-Mortem							Difference (0 to 24 Hours)	
	0	0.5	1	2	3	4	24	ΔpH	$\Delta[H^+]$ moles $\times 10^{-7}$
Age	[b]				[b]	[b]	[b]	[a]	
125 days	7.03	6.76	6.60	6.52	6.46	6.42	6.19	0.85	6.33
175 days	7.14	6.77	6.72	6.58	6.48	6.38	5.85	1.29	14.44
225 days	7.35	6.76	6.65	6.47	6.24	6.01	5.69	1.66	20.45
Difference[a]	0.10	...	...	...	0.16	0.14	0.11	0.12	...
Breed									
Hampshire	7.16	6.84	6.71	6.58	6.45	6.33	5.90	1.27	14.69
Chester White	7.18	6.69	6.60	6.47	6.33	6.21	5.93	1.26	12.80
	0.02	0.15	0.11[a]	0.11	0.12	0.12[a]	0.03	0.01	1.89
Layer									
Outer	7.27	6.85	6.73	6.58	6.44	6.33	5.97	1.31	12.08
Inner	7.08	6.67	6.58	6.47	6.34	6.20	5.85	1.22	15.40
	0.19[b]	0.18[b]	0.15[b]	0.11	0.10	0.13[a]	0.12[a]	0.09	3.32[b]
Age × Breed		[a]		[a]				[a]	[b]

[a] $P < 0.05$.
[b] $P < 0.01$.

pH owing to increasing animal age were observed, while the 3-, 4-hour, and ultimate pH values decreased with age. Greater changes in ΔpH occurred as the animals increased in age. However, no important ($P > 0.05$) age effect on $\Delta[H^+]$ was noted because of the highly significant age-times-breed interaction resulting from inconsistent breed differences from age to age. Hampshire animals had higher 1- and 4-hour pH values than the Chester Whites. Highly significant and significant layer differences, following the same pattern as previously reported (Sink, 1966) were noted. The outer layer of the *panniculus adiposus* always exhibited a higher pH but the $\Delta[H^+]$ between initial and ultimate values was greater in the inner layer. Significant age-times-breed interactions were noted for the 0.5-, 2-hour, and ΔpH measurements; a highly significant age-times-breed interaction was noted for $\Delta[H^+]$.

DISCUSSION

Adipose tissue, once regarded as metabolically inert, is now recognized to contain enzymes catalyzing a wide variety of synthetic and degradative processes. Studies of lipid metabolism in adipose tissue have shown that aging results in a decrease in both oxygen uptake (Ball *et al.*, 1959) and lipid biosynthesis (Benjamin *et al.*, 1956). Sorokin (1964) has suggested that with biosynthetic enzymes, destroyed or inactive, the size of the metabolic mechanism rapidly declines. Catabolic activity increases and anabolic activity decreases with age to the extent that the decline in metabolic rates is continuous. Perhaps this may explain not only the change but also the continuous increase in tissue pH with increasing age. Fatty acid desaturation mechanisms decrease with increasing age (Sink *et al.*, 1964). The question remains, does the changing metabolic pattern(s) result in pH changes or do pH changes influence metabolic shifts? Genetic or breed effects on tissue pH may be related to an inherent difference in the biosynthetic capacity of the *panniculus adiposus* between these breeds. In this study, Chester Whites were more obese (greater average subcutaneous fat thickness) than the Hampshires.

Benjamin *et al.* (1956) suggests that a contributing factor to obesity with aging may be a sluggish response to physiologic catalytic stimuli in the older animals. This sluggish response may be related to a decreased ability to incorporate free fatty acids into tissue lipid. The glycerol phosphate required for glyceride synthesis is usually available from glucose through dihydroxyacetone phosphate. Bally *et al.* (1960) have suggested that in the absence of glucose and in the presence of free fatty acids, esterification (and thus incorporation of the free fatty acids into tissue lipid) is limited by the capacity of the tissues to produce glycerol phosphate. Perhaps this explains both the age and breed effects on ΔpH and/or $\Delta[H^+]$.

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