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Collagen Composition of the Skin from Obese and Lean Zucker Rats (40071)

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The genetically obese Zucker rat transmits obesity as a Mendelian recessive trait (1). This rodent is frequently studied as a model of juvenile-onset obesity in humans because both obese Zucker rats (2) and certain types of obese humans (3) demonstrate many similar metabolic, endocrinologic and morphologic abnormalities. Although major differences in lipid metabolism between obese and lean animals and humans have been reported, possible alterations in protein metabolism in obesity are much less well defined.

Significant deviations in growth between obese and lean Zucker rats occur at an early age, and fat continues to accumulate progressively throughout life (4, 5). Carcass analyses support these observations; obese rats demonstrate significant increases in body fat and decreases in carcass protein and water compared to their lean littermates, even under pair-feeding conditions (6, 7). Obese rats are inefficient in their utilization of dietary nitrogen when compared to lean rats; less nitrogen is converted to body protein and more nitrogen is excreted by the obese (8, 9). Physical exercise increases the body protein content of the obese Zucker rat but not to the level of the lean (10). An impairment in growth hormone production has been suggested as an explanation for the generation of stunting and obesity in the obese Zucker rat (4). Growth hormone deficiency in children retards growth and leads to increased deposition of body fat; growth hormone administration reverses these effects (11). Obese humans demonstrate a significantly blunted response to stimuli which increase growth hormone levels in lean subjects, e.g., exercise (12), arginine (13, 14) and l-dopa (15, 16).

Altered protein metabolism in obesity is also suggested in studies of protein synthesis and amino acid levels. Obese Zucker rats demonstrate increased levels of hepatic protein synthesis (17, 18), however, the protein content of livers from obese rats appears to be reduced compared to hepatic protein levels in lean rats (18). Circulating levels of leucine, isoleucine, valine, phenylalanine and tyrosine are elevated and glycine is reduced in obese compared to lean subjects (19).

The present study was designed to investigate collagen metabolism in skin of lean and obese Zucker rats. This protein and tissue were selected because of (a) the ubiquitous and abundant occurrence of collagen, and (b) the observation that the skin of obese rats tears and wounds more easily than the skin of lean animals (20). The results reported here indicate a normal collagen composition but an abnormal accumulation of lipid in the skin of obese Zucker rats. The reduced break strength of skin from obese rats, due probably to the decreased amount of collagen per cm^2 caused by a displacement of collagen fibers by lipid, may explain the frequent skin tearing.

Materials and Methods. Animals. Genetically obese rats and lean littermates purchased from L. M. Zucker (Harriet G. Bird Memorial Laboratory, Stow, MA) were housed in wire-bottomed cages in a temperature-regulated (22°) light-controlled room. Animals were fed Purina Laboratory Chow *ad libitum* prior to sacrifice.

Determination of skin collagen. The animals were killed by decapitation, their backs were shaved, a section (approximately 8 × 5 cm) of skin was removed from the midportion of the back and then the skin was freed of adhering subcutaneous tissues. Eight punches were made through the full thickness skin using a No. 8 cork borer. The skin punches were divided randomly into two equal samples, the wet weights of the samples were recorded, and then the samples of skin were dried by lyophilization. After recording the dry weight, the pieces of skin were extracted overnight at room temperature with 20 ml of chloroform–methanol (2:1). The pieces of skin were dried under a stream of nitrogen,

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and then the lipid-free dry weight was recorded. The skin samples were hydrolyzed at 105° for 24 hr in 5 ml of 6 *N* HCl. The amount of hydroxyproline in the hydrolysate was determined using a Technicon autoanalyzer (21). The hydroxyproline values were multiplied by 7.23 to obtain collagen equivalents (22).

Extraction and characterization of skin collagen. Skin samples (1–1.5 g) were minced and then shaken with 10 ml of 0.5 *M* acetic acid for 24 hr at 4°. Insoluble material was collected by high speed centrifugation, then reextracted. The supernatants were removed by decantation. The combined supernatants were dialyzed exhaustively against 0.05 *M* acetic acid, then dried by lyophilization. The skin residue after acid extraction was treated with pepsin according to the procedure of Chung and Miller (23). The pepsin-solubilized collagen was dialyzed at 4° against 0.15 *M* phosphate buffer (pH 7.6) and then against the same buffer containing 1.5 *M* NaCl. The precipitate which formed during dialysis was collected by centrifugation. The pellet was redissolved and the precipitation with 1.5 *M* NaCl repeated. The supernatants from the first and second precipitations were combined. The pellet was dissolved in 0.5 *M* acetic acid and then the dissolved pellet and the supernatant were dialyzed against 0.05 *M* acetic acid. Following dialysis, the samples were dried by lyophilization. Differential salt precipitation (23, 24) of pepsin-solubilized collagen yields a fraction enriched in Type III collagen (1.5 *M* NaCl-insoluble) and a fraction enriched in Type I collagen (1.5 *M* NaCl–soluble).

Break strength of skin. A section of back skin was removed and cleaned of hair, subcutaneous fat, and fascia. The full thickness skin was cut into strips 1.25 cm wide and the break strength of the skin measured using an Instron Universal Testing Instrument (Canton, MA) equipped with 100 kg tension load cell.

Results and discussion. Composition of the skin. Data concerning the chemical composition of skin from obese and lean Zucker rats are presented in Table I. The skins from the obese animals contained significantly more lipid per g wet weight than did the skins from their lean counterparts, even though all skins were free of visible subcutaneous fat and fascia. This effect which was more pronounced in females than in males was also more pronounced in the younger animals than in the older animals. The amount of water and of lipid-free dry residue per g wet weight was decreased in the obese skins when

TABLE I. COMPOSITION OF SKIN FROM OBESE AND LEAN ZUCKER RATS.

			Skin Composition[a]		
Animals	Age	Body weight	% H_2O	% Lipid	% Lipid-free dry-residue
Female lean	2 months (6)	170 ± 4	68.3 ± 1.6	8.2 ± 1.0	24.6 ± 0.4
	6 months (7)	—	59.8 ± 0.6	4.5 ± 0.3	35.6 ± 0.4
Female obese	2 months (6)	235 ± 8	41.2 ± 1.6[b]	41.9 ± 2.1[b]	16.8 ± 0.6[b]
	6 months (7)	—	42.5 ± 3.8[b]	36.1 ± 4.1[b]	21.4 ± 1.4[b]
Male lean	3 months (5)	256 ± 12	65.9 ± 2.0	6.7 ± 2.1	27.1 ± 0.7
	6 months (7)	—	61.0 ± 0.6	5.4 ± 0.4	33.5 ± 0.3
Male obese	3 months (4)	340 ± 12	46.5 ± 3.4[b]	34.4 ± 3.7[b]	19.1 ± 0.5[b]
	6 months (7)	—	53.9 ± 2.6[b]	18.5 ± 2.7[b]	27.6 ± 0.9[b]

[a] Percentage values for skin composition are expressed as percent of wet weight. The numbers in parentheses represent the number of animals in each group. Means ± SE of the mean are reported.

[b] These values for the skins from obese animals are significantly different from the values for the skins from age and sex matched lean animals at the level of $P < 0.05$ (Student's *t* test).

compared to lean skins. These data suggest that there may be an atypical infiltration of lipid into the skin of the obese Zucker rat.

Collagen content of skin. Table II shows the data comparing the collagen content of skin from obese Zucker rats with the collagen content of skin from age and sex matched lean Zucker rats. When the collagen values were expressed as mg collagen per g wet weight or per g dry weight, the skins from the obese rats contained significantly less collagen than the skins from the lean animals. However, when the collagen values were expressed as mg collagen per g lipid-free dry weight, all differences in the collagen content of skins from lean and obese animals disappeared. On a mg collagen per cm^2 of skin basis, the skins from the obese animals again contained significantly less collagen than the skins from lean animals. These data demonstrate that the ratio of collagen to noncollagen dry residue (lipid-free) is the same in skins from lean and obese animals. Therefore, the apparent decrease in the collagen content (mg collagen per g wet or dry weight) of the skins from obese Zucker rats probably results from an abnormal accumulation of lipid in the skin, rather than from a defective deposition of skin collagen.

Properties of collagen from skin of obese and lean Zucker rats. The data in Table III demonstrate that the ratio of acid soluble to acid insoluble collagen was the same for skins from obese and lean animals of either sex. There was a slight but significant increase in the amount of pepsin-solubilizable collagen in the skin of obese females when compared to lean females. No such increase was found for the male animals. The ratio of 1.5 *M* NaCl-soluble (Type I enriched) to 1.5 *M* NaCl-insoluble (Type III enriched) collagen in the pepsin soluble fraction was the same for all groups of animals. These data taken together with the data in Tables I and II suggest that the collagen of obese skins is essentially normal and not different from the collagen of lean skins when compared on the basis of quantity per g lipid-free dry weight, on the basis of solubility in acid, or on the basis of the ratio between 1.5 *M* NaCl-soluble and 1.5 *M* NaCl-insoluble collagen. The data demonstrating that the obese skins contain less collagen per cm^2 of full thickness skin than the lean skins suggest either that the obese skins may be thinner or that the density of collagen per cm^2 of obese skin is decreased because of displacement of collagen fibers due to the abnormal accumulation of lipid.

Break strength of skin. The break strength of skin from obese animals was significantly

TABLE II. COLLAGEN CONTENT OF SKIN FROM OBESE AND LEAN ZUCKER RATS.

		Collagen content of skin[b]			
Animals	Age[a]	mg collagen/g wet wt. skin	mg collagen/g dry wt. skin	mg collagen/g lipid-free dry wt. skin	mg collagen/cm^2 skin
Female lean	2 months (6)	151.4 ± 4.5	463.6 ± 21.2	616.2 ± 10.5	—
	6 months (7)	262.5 ± 7.8	656.0 ± 21.0	738.9 ± 23.6	26.1 ± 1.4
Female obese	2 months (6)	106.8 ± 3.0[c]	182.4 ± 8.1[c]	636.8 ± 26.4	—
	6 months (7)	158.4 ± 20.1[c]	301.6 ± 63.3[c]	728.0 ± 26.3	22.5 ± 0.7[c]
Male lean	3 months (5)	159.9 ± 8.2	476.1 ± 39.2	589.3 ± 22.6	—
	6 months (7)	248.9 ± 5.4	640.6 ± 15.5	743.2 ± 11.3	39.4 ± 2.0
Male obese	3 months (4)	117.5 ± 3.4[c]	222.7 ± 18.2[c]	615.2 ± 11.4	—
	6 months (7)	205.4 ± 12.0[c]	463.6 ± 50.8[c]	743.1 ± 13.1	29.4 ± 1.2[c]

[a] The numbers in parentheses represent the number of animals in each group.

[b] Means ± SE of the mean are reported.

[c] These values for the skins from obese animals are significantly different from the values for the skins from age and sex matched lean animals at the level of $P < 0.05$.

TABLE III. PROPERTIES OF COLLAGEN FROM THE SKIN OF OBESE AND LEAN ZUCKER RAT.[a]

Animals	mg Collagen/100 mg recovered collagen[b]			Pepsin-solubilized collagen[b] sol. in 1.5 *M* NaCl
	Acid soluble	Pepsin soluble	Insoluble	pepsin-solubilized collagen ppt. by 1.5 *M* NaCl
Female lean (6)	11.3 ± 0.7	31.4 ± 1.2	57.3 ± 1.3	5.8 ± 0.4
Female obese (4)	12.4 ± 0.8	38.0 ± 1.0[c]	49.6 ± 1.8[c]	5.2 ± 0.2
Male lean (6)	15.4 ± 1.2	35.8 ± 2.1	48.7 ± 3.2	5.8 ± 0.4
Male obese (4)	16.7 ± 1.8	33.4 ± 1.3	49.9 ± 2.7	5.8 ± 0.2

[a] These studies were done on skins from animals sacrificed at 6 months of age. The numbers in parentheses represent the number of animals in each group.

[b] Means ± SE of the means are reported. The recovery of collagen (acid sol. + pepsin sol. + insoluble/total collagen) averaged 83.3% with a range from 71.6% to 108.8%.

[c] $P < 0.05$.

TABLE IV. BREAK STRENGTH OF SKIN FROM OBESE AND LEAN ZUCKER RATS.

Animals	Break strength[a] (kg)
Female lean (3)	10.7 ± 0.7
Female obese (3)	8.0 ± 0.6[b]
Male lean (3)	17.1 ± 1.1
Male obese (3)	12.7 ± 0.7[b]

[a] Animals (males, 8 months old; females 9 months old) were sacrificed and strips of back skin 1.25 cm wide prepared. The break strength of the skins was measured as described in Materials and methods. Means ± SE of the mean are reported. Number in parentheses represent the number of animals in each group.

[b] These values for the obese animals are significantly different from the values for age and sex matched lean animals at the level of $P < 0.05$.

lower than the break strength of skin from lean animals (Table IV). These data are in agreement with those demonstrating a decreased amount of collagen per cm^2 of obese skin since the break strength of skin is, in general, a function of collagen content per unit area (25). The decreased break strength of the obese skins may explain the observation that the skin of obese animals tears and wounds more easily than the skin of lean animals.

Summary. The skins of obese Zucker rats contained per g wet weight significantly more lipid and significantly less water than did the skins from age and sex matched lean Zucker rats. The obese skins also contained less collagen than the lean skins when compared on the basis of mg collagen per g wet weight, per g dry weight, or per cm^2 of skin; however, no difference was found when the skins were compared on the basis of mg collagen per g lipid-free dry weight. The solubility properties of the collagen from obese and lean skins were the same while the break strength of the obese skins was decreased. The results indicate that skins from obese Zucker rats have a normal collagen composition but an abnormal lipid composition. The reduced break strength of the skin from obese rats, due probably to the decreased amount of collagen per cm^2 of skin caused by a displacement of collagen fibers by lipid, may explain the frequent skin tearing observed in the obese rats.

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