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Mechanical and chemical properties of the skin and its collagen from lean and obese-hyperglycaemic *(ob/ob)* mice

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Summary. We have compared the mechanical and chemical properties of the skin and its collagen from 24-week-old obese-hyperglycaemic *(ob/ob)* and lean mice. The skin from obese mice was mechanically weaker and generated a lower hydrothermal isometric force. However, there were no significant differences from lean mice in the type of reducible cross-links in the collagen or in its solubility, although it contained more reducible cross-links and glycosylated lysine. The total amount of skin collagen was similar in obese and lean mice from 3 to 24 weeks of age but the skin surface area was 60% greater in 24-week-old obese mice. When corrected for collagen content the tensile strength of skin from obese mice was greater than that from lean mice and we suppose that the weakness of obese mouse skin is caused by a failure of collagen deposition to match the increase in skin surface area as the animals become obese.

Key words: Collagen, *(ob/ob)* mice, obese-hyperglycaemic mice, skin, tensile strength, cross-links, solubility, glycosyllysine, surface area.

Diabetes mellitus causes changes in the structure and metabolism of interstitial and basement membrane collagens [1–3]. Studies on animals treated with alloxan or streptozotocin suggest that diabetes increases the cross-linking within the collagen molecule, as evidenced by decreased solubility [4–5] and susceptibility to collagenases [6], and may increase the size and stiffness of tendon fibres [7]. Increased cross-linking and stiffness of collagen occur during the normal ageing process and it has been suggested that diabetes may therefore cause accelerated ageing [6–8] but there is no increase in the conversion of reducible to non-reducible cross-links which characterizes normal ageing [9–10]. The increased non-enzymic glycosylation of collagen [11–12], that occurs as a result of diabetic hyperglycaemia, has been implicated in the increased stability of collagen from diabetic rats but the mechanism of this stabilisation is not clear [9–10]. Obese-hyperglycaemic *(ob/ob)* mice resemble maturity onset, non-insulin-dependent diabetes, having mild fasting hyperglycaemia, hyperinsulinaemia and insulin resistance [11] and would be expected to show similar changes in the physical and chemical properties of their collagen. However, contrary to such expectations, we observed a weakness in their skin, similar to that reported in fatty *(fa/fa)* rats [13]. We have therefore investigated the mechanical strength of skins from obese and lean mice and related this to the skin composition and the cross-linking and glycosylation of the collagen.

Materials and methods

Obese-hyperglycaemic *(ob/ob)* mice were from our own colony derived in turn from the C57Bl/6J-*ob* stock of the Jackson Laboratory, Bar Harbor, Maine, USA. The lean controls were from a sub-colony from which the obese *(ob)* gene had been selected out.

The skin from the torso region, the proximal region of limbs and neck of female mice aged 24–26 weeks was used for the investigation unless otherwise stated. Loosely associated fat but not the fur was removed from the skins.

Skin composition

The surface area and composition of skins from individual animals of 3–48 weeks of age were determined. The skins were dissected as before and laid flat on a plastic sheet, the periphery was traced and the area determined using a planimeter (Allbrit, from Stanley, London, UK). The skins were bisected longitudinally at the midline and one-half was freeze-dried before the fat was extracted in a Soxhlet extractor (Corning, Stone, UK) with cholorform: methanol (2:1, v/v) for 6 h. After drying and re-weighing, the collagen content of this fat-free dry matter was determined by hydrolysing in constant boiling hydrochloric acid (6 N) at 112 °C for 24 h and then measuring the hydroxyproline content by the method of Grant [14] using a Technicon Autoanalyser (Technicon Corporation, Audsley, New York, USA). Small samples of skin were hydrolysed without drying or lipid extraction.

Table 1. Results of mechanical testing of skin from 24-week-old female lean and obese *(ob/ob)* mice

	ob/ob mice	Lean mice
Collagen concentration (mg/cm^2 surface at test site) ($n=18$)	2.1 ± 0.4	4.0 ± 0.3^a
Maximum tensile force (N) ($n=6$)	2.8 ± 0.1	4.1 ± 0.3^a
Ultimate tensile strength, (N/cm^2 cross-sectional area) ($n=6$)	45.9 ± 1.6	107 ± 5.9^a
Maximum force (N/mg collagen per cm) ($n=6$)	2.62	2.04
Maximum thermal isometric tension ($N \times 10^3$) ($n=12$)	31.9 ± 1.4	77.5 ± 5.5^a
Isometric tension ($N \times 10^3$/mg collagen per cm) ($n=12$)	30.4	38.8

Results are expressed as mean ± SEM for the number of animals shown in parentheses. $^a p < 0.001$, values differ significantly between lean and obese mice

Skin tensile testing

The tensile strength of strips of skin from the dorsal surface of lean and obese mice was tested on an Instron TM-SM materials testing machine (Instron, HighWycombe, Bucks, UK). The strips (20 × 5 mm) were cut across the dorsal axis so the axis bisected the strip. Three strips per skin from 12 lean and 11 obese animals were stretched at 10 mm/min at 20 °C in NaCl solution (0.154 mol/l). In each case the maximum force withstood prior to heating was measured [15] and the ultimate tensile strength was calculated using the original cross-sectional area of the strips.

An attempt was made to improve measurements of the dimensions and density of the strips by using the chemical depilation procedure of Crews et al. [16].

Skin hydrothermal isometric tensile testing

For these tests we used an apparatus and method similar to that described by Allain et al. [17]. Strips of skin (15 × 5 mm) from six lean and six obese mice were prepared as for the tensile testing and held under slight tension between two jaws whilst immersed in physiological saline. The saline was heated at 3.8 °C/min and as the strip contracted the force generated was measured by a transducer (Sangamo Transducers, Bognor Regis, UK) attached to the upper jaw. The mean force generated per square cm was calculated for two strips from each mouse. The collagen content was determined either on strips taken from the opposite side of the body of obese mice or from the same site on skins from lean mice of the same age and sex.

Skin collagen solubility

Fresh lean and obese skins, from at least three mice of each type, were pooled and minced in an ice-cold domestic hand mincer. Weighed replicate aliquots of the minced skins were added to 20 volumes of distilled water at 80 °C and stirred at this temperature for 2 h. The insoluble and soluble fractions were separated by centrifugation at 10,000 *g* for 30 min, freeze-dried and the collagen content of each determined as described above.

Reducible components in collagen

Replicate aliquots (1g) of fresh, minced, pooled skins were washed overnight in cold physiological saline. They were then reduced, in fresh saline, with tritiated KBH_4 (0.3 μCi/mg) for 1 h at room temperature [18]. The saline was adjusted to pH 4 with acetic acid and the sample dialysed overnight against running cold tap water. After freeze-drying, the sample was weighed and hydrolysed as before. The hydrolysate was chromatographed on Zeocarb 225 (BDH Chemicals, Poole, Dorset, UK) using the volatile solvent system described by Bailey et al. [19] and the radioactive components detected in an LKB Rackbeta scintillation counter (LKB, Selsdon, Surrey, UK) using Packard Instagel (Packard Instruments, Caversham, Berks, UK). The counts incorporated in each component were corrected for the amount of collagen reduced, calculated from the hydroxyproline content.

Blood glucose determination

Samples of blood (20 μl) were obtained from the retro-orbital plexus of fed mice under ether anaesthesia between 11.00 and 13.00 h. After deproteinization by the barium hydroxide/zinc sulphate procedure glucose was measured by the glucose oxidase technique using 2.2'-azino-di-(3-ethyl-benzthiazoline sulphonate(6)) (Boehringer, Lewes, Sussex, UK) as the chromophore.

Statistical methods

Results are expressed as mean ± SEM. Comparisons between obese and lean mice was by one-tailed Student's t-test and $p < 0.05$ was considered significant.

Results

Tensile strength and isometric tension

Measurements of tensile strength confirmed our original observation that far from being stronger than lean controls, the skins of obese mice were much weaker (Table 1). The maximum strength of strips from obese mice was only 68% of strips from lean skins. The ultimate tensile strength, expressed as force per cross-sectional area, of obese mouse skin was still less, at 43% of that for lean skin because of the greater thickness of the obese skin. To improve the accuracy of determination of the dimensions of the strips of skin, some carcasses were treated with a chemical depilatory before removal of the skin. This procedure did not decrease the maximum tensile force but it caused swelling of the skin with a marked increase in thickness so that the calculated tensile strength was less by 28% for obese skins and 15% for lean skins compared with untreated skin. The treatment was therefore discontinued and, since it indicated that the maximum force was independent of skin thickness, maximum force was related to the collagen concentration on the assumption that it is the major component responsible for the tensile strength of skin. The collagen content of the sample strips from obese mice was only 53% of that of strips from lean mice (Table 1) so that the maximum force per mg of collagen per cm was 28% greater for obese skin than lean skin.

The skin strength was also determined by measuring

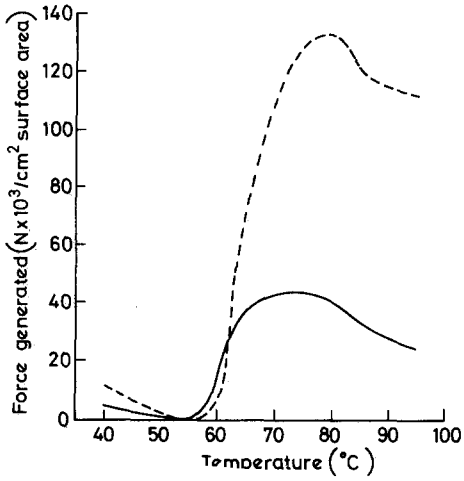

Fig. 1. Hydrothermal isometric contraction/relaxation curves for typical samples of skin from an obese (—) and lean mouse (– – –)

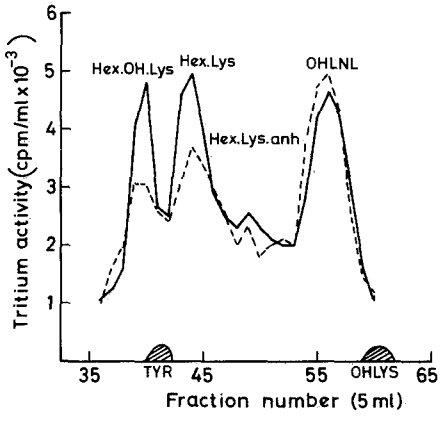

Fig. 2. Elution order from Zeocarb 225 of the radiolabelled components of lean (– – –) and obese (___) mouse skins after reduction with ^3H borohydride and acid hydrolysis. Hex. OH lys., hexosyl hydroxylysine; Hex. lys., hexosyllysine; Hex. lys. anh., hexosyllysine anhydride; OH LNL; hydroxsyllysinonorleucine; Tyr., tryosine; OH lys., hydroxsyllysine

the isometric tension generated by thermal denaturation of the collagen. The maximum thermal tension developed by skin from obese mice was only 41% of that produced by heating lean skins (Table 1) and for equal weights of collagen, the obese skins remained weaker than the lean skins, producing only 79% of the tension of the latter. Despite this weakness, the pattern of the development and maintenance of thermal tension was similar in lean and obese skin (Fig. 1), suggesting that there were no differences in the types of cross-links present in the collagen.

Skin collagen solubility

The solubility of collagen depends upon the type and number of cross-links. Although the amount of collagen solubilized by heating minced skin in water at 80 °C for 2 h was less for obese than lean mice (Table 2) the percentage of the total collagen solubilized was the same for each type of skin.

Table 2. Solubility of the skin collagen from 24-week-old female lean and obese *(ob/ob)* mice

	ob/ob mice	Lean mice
Solubilised collagen (mg)	6.8 ± 0.9	13.7 ± 3.0
Insoluble collagen (mg)	13.1 ± 1.3	25.4 ± 2.4
Total collagen (mg)	19.8	39.1
Collagen solubilised (%)	34	35

Results are expressed as mean ± SEM for three replicate determinations on three pooled samples from each type of mouse

Table 3. Radiolabelled components from the skin of 24-week-old female lean and obese *(ob/ob)* mice, after reduction with ^3H potassium borohydride

	ob/ob mice	Lean mice
Blood glucose (mmol/l)	11.0 ± 0.7	8.8 ± 0.1
Total ^3H incorporated into skin (cpm × 10^{-5}/g)	1.74 ± 0.05	2.29 ± 0.25
Total ^3H incorporated/mg collagen (cpm)	2120 ± 57	1251 ± 139
Hexosylhydroxylysine (cpm/mg collagen)	131.3 ± 6.0	48.3 ± 3.5
Hexosyllysine (cpm/mg collagen)	221.4 ± 6.0	71.9 ± 4.5
Hydroxylysinonorleucine, (cpm/mg collagen)	131.5 ± 3.7	93.9 ± 6.4

Results are expressed as mean ± SEM for three replicate determinations on skins pooled from six mice in each group, except for blood glucose which is for nine mice in each group

Reducible components of collagen

Because of the contradictory nature of our findings, in relation to the greater strength but unchanged solubility of skin collagen from obese mice, we determined the labile aldimine cross-links present by reduction with tritiated borohydride. This procedure also reduces the aldimines formed by non-enzymic glycosylation of collagen and, since increased glycosylation in diabetes has been implicated in changes in the characteristics of collagen, we measured the incorporation of tritium into hexosyllysines. Samples of obese skin incorporated only 76% as much total radioactivity as equal weights of lean skins (Table 3) but incorporated 70% more per mg of collagen. Figure 2 shows the position of the reduced components as they are eluted from the ion exchange column, and also the relative amounts prior to correction for differences in collagen content. The hexosyllysine anhydride shown on the chromatogram is formed from hexosyllysine during the hydrolysis of the skin. Qualitatively there were no differences in the components present in the hydrolyses from reduced lean and obese skin. However, the obese skin collagen contained

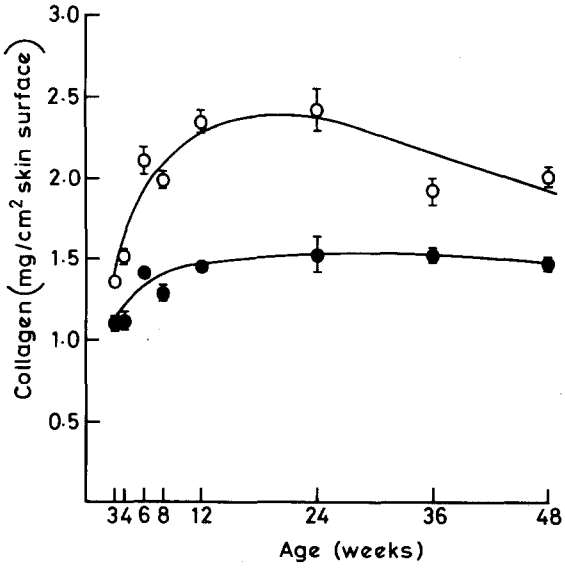

Fig. 3. The effect of age on the collagen content (mg/cm^2 surface area) of lean (○) and obese (●) mouse skins. The differences at each age were significant by Student's t-test ($p < 0.01$)

two-to-threefold more glycosylated lysine and hydroxylysine than the lean skin and 40% more reduced collagen cross-links. The blood glucose concentration was 25% higher in the obese mice compared with the lean mice (Table 3).

Influence of age on skin composition

Between 3–24 weeks of age the total quantity of skin collagen increased in a similar manner in lean and obese mice and continued to increase up to 48 weeks in obese mice. In lean mice it reached a plateau between 24–48 weeks so that they contained significantly less than obese mice at 36 and 48 weeks (Table 4). At 3 weeks the skin surface area of obese mice was 13.5% greater than that of lean mice ($p < 0.05$). Although the surface area of both increased up to 48 weeks, the difference increased also and at 48 weeks obese skins were 93% larger than lean skins ($p < 0.001$; Table 4). At 3 weeks of age the weight of collagen per cm^2 of skin was significantly lower in obese skins (82% of the concentration in lean skins $p < 0.001$; Fig. 3). The amount of collagen per unit area of skin increased up to 12 weeks in both lean and obese mice as also did the difference between them. In obese mice there was little further change up to 48 weeks but in lean mice it decreased slightly, so that the greatest differences were between 12 and 24 weeks.

The proportion of fat reached a maximum of 69% at 12 weeks in obese mice and 45% at 36 weeks in lean mice. The proportion of fat-free, dry matter was lower in skins from obese mice than lean mice at 3 weeks and decreased slowly and irregularly in both (Table 4). However, as a result of their greater weight, the skins of obese mice over 8 weeks of age contained a greater quantity of fat-free dry matter.

Influence of age on blood glucose concentration

At 3 weeks the blood glucose concentration of obese mice was 21% lower than that of lean mice ($p < 0.001$). Subsequently it increased to a peak 54% above that in lean mice at 8 weeks and then declined to normal concentrations at 48 weeks.

Discussion

These results show that the skin of obese mice is weaker than the skin of lean mice because of its low collagen concentration. However, because of reports of increased stability [4–6], stiffness [7] and strength [9] of collagen in diabetes, we looked for similar changes in the skin collagen of obese mice. When calculated on the basis of collagen content, the obese skin was 28% stronger than lean skin. The unexpectedly low thermal isometric tension expressed as Newtons/mg collagen per cm^2 of obese mouse skin probably arises because the collagen fibres dispersed in the thick fatty layer are more able to undergo realignment to relieve the tension generated during heating. However, the forces applied during tensile testing are much greater and realignment would be completed well before the breaking point.

Table 4. Effect of age on the composition of skin from obese and lean mice

Age of mice (weeks)	Weight of mice (g)		Blood glucose (mmol/l)		Weight of skin (g)		Area of skin (cm^2)		Fat (%)		Fat-free dry matter (%)		Collagen (mg)	
	Obese	Lean	Obese	Lean	Obese	Lean	Obese	Lean	Obese	Lean	Obese	Lean	Obese	Lean
3	10.1±0.2 (5)	8.1±0.4 (6)	6.8±0.1	8.6±0.3	0.92±0.02	0.60±0.04	31.9±0.7	28.1±1.5	44.6±1.4	19.8±0.6	20.1±0.6	28.9±0.6	35.2±1.0	38.2±2.3
4	14.8±0.8	10.4±0.8	8.4±0.4	9.1±0.2	1.18±0.07	0.82±0.11	40.0±1.6	31.2±1.3	48.0±1.7	14.7±1.1	17.6±0.7	27.4±1.3	44.5±2.7	47.3±4.4
6	27.0±1.0	17.5±0.4	9.8±0.7	8.8±0.2	2.49±0.10	1.85±0.10	61.0±1.6	47.3±1.9	50.7±2.9	23.1±1.6	14.4±1.3	26.9±0.7	85.9±3.6	100.1±7.3
8	37.7±0.8	19.4±0.3	12.5±0.6	8.1±0.2	3.16±0.09	1.79±0.06	73.6±2.2	48.2±1.1	55.4±1.0	20.0±1.1	19.2±1.1	29.4±1.4	94.0±5.1	95.6±2.2
12	47.6±0.6	20.9±0.5	12.6±0.9	8.5±0.1	4.20±0.10	1.97±0.07	86.6±1.7	53.3±1.7	69.3±0.5	30.3±1.7	12.6±0.5	27.9±0.8	126.3±1.3	124.7±3.5
24	61.4±1.6 (10)	28.5±0.9 (10)	8.8±0.5	7.9±0.2	6.20±0.18	2.84±0.10	95.3±4.2	58.3±2.7	61.3±1.3	37.0±0.8	19.3±1.2	25.6±0.6	141.9±4.2	125.6±3.2
36	69.9±3.0	30.4±1.0	9.5±0.5	8.0±0.3	6.18±0.25	2.78±0.23	116.4±4.3	66.8±2.7	65.7±1.1	44.7±2.4	14.7±0.6	22.9±1.6	173.6±5.2	127.5±3.9[a]
48	80.1±2.7	30.7±1.9	8.1±0.3	ND	6.69±0.32	2.91±0.18	133.5±4.8	69.0±1.9	61.7±0.5	39.3±3.0	15.1±0.4	23.5±1.4	192.2±8.2	138.0±2.9[a]

Results are expressed as mean±SEM for six obese and six lean mice except where otherwise indicated in parentheses. [a] $p < 0.001$; significant difference between corresponding parameters for lean and obese mice; ND = not determined

The greater tensile strength of collagen in skin from obese mice could arise from the greater glycosylation which is known to increase tensile strength in collagen of streptozotocin-induced diabetic rats [9], although the mechanism of this effect is not understood. Increased non-enzymatic glycosylation [20, 21] stems from the hyperglycaemia of the mice used for this part of the study. Two years later, when the effect of age on the development of skin collagen was investigated, the 24-week-old mice were no longer hyperglycaemic. This is in line with a gradual decrease in the hyperglycaemia of the older mice in our colony over the last 8 years but because of the long half-life of collagen, increased glycosylation at any earlier age would be retained.

The 40% increase in reducible cross-links indicated by tritium incorporation through reduction of the C = N bond of dehydrohydroxylysinonorleucine and/or dehydrohydroxylysinohydroxynorleucine would also contribute to the increased tensile strength of obese skin collagen. Since increased labile cross-links have not been observed in tail tendon collagen of streptozotocin-diabetic rats [9, 10], it is not clear whether there is a difference between the tissues or whether the difference results from the obesity and not the diabetic state. Apparent differences in labile cross-links might also be artefacts due to differences in the accessibility of the reducible sites to the reagents, but there is no evidence that this occurs.

The unchanged solubility of skin collagen from obese mice appears contrary to reports of decreased solubility in diabetes [4, 5]. This discrepancy arises from differences in the methodology. Heating at 80 °C for 2 h extracts all collagen which lacks the stable, non-reducible cross-links present in older animals [22, 23] and was used to test for premature ageing which was found to be absent. Had we used the less rigorous tests of solubility [4, 5] which do not completely disrupt labile cross-links, we would expect to have observed decreased solubility caused by both increased glycosylation and labile cross-linking.

The normal deposition of skin collagen of obese mice up to 24 weeks of age clearly differs from the loss of collagen from skin of insulinopenic streptozotocin-diabetic rats which results from a decrease in synthesis [24]. However, not all insulin-dependent processes are decreased in obese mice, which are better models for non-insulin-dependent rather than insulin-dependent diabetes. It also seems unlikely that a tendency to decreased synthesis is exactly compensated by an obesity-induced increase in synthesis. However, the continued collagen deposition in obese mice after 24 weeks is evidence of a continuing stimulus but decreased collagen deposition occurs in sponges implanted subcutaneously into obese mice compared with lean mice [25]. This latter defect resembles the poor wound-healing observed in non-insulin-dependent diabetic patients but it's relevance is doubtful because of the different regulatory processes and physiological state of the animals during wound healing and normal growth. The presence of similar total amounts of collagen in lean and obese mouse skin is perhaps surprising in view of the elevated corticosteroids in obese mice [26, 27] which are known to inhibit collagen synthesis and production of enzymes involved in collagen synthesis [28].

Although we have established that the weak skin of obese mice results from its low collagen concentration, it is not clear whether the diabetic state or obesity is the cause. However, skin collagen is more glycosylated and is stronger in agreement with other studies of diabetes. Further work is required to seperate the effects of increased surface area per se from hyperinsulinaemia and hypercorticism, particularly with regard to the quantity of skin collagen and the novel observation of increased labile cross-links.

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References

1. Francis MJO, Ellis JP, Hockaday TDR (1974) Skin collagen in diabetes mellitus in relation to treatment. Proc Roy Soc Med 67: 35–36
2. Cohen MP, Vogt CA (1975) The effect of diabetes on renal lysine utilization. Horm Metab Res 7: 338–342
3. Brownlee M (1976) α2-Macroglobulin and reduced basement-membrane degradation in diabetes. Lancet 1: 779–780
4. Golub LM, Garant PR, Ramamurthy NS (1977) Inflammatory changes in gingival collagen in the alloxan-diabetic rat. J Periodont Res 12: 402–418
5. Golub LM, Greenwald RA, Zebrowski EJ, Ramamurthy NS (1978) The effect of experimental diabetes on the molecular characteristics of soluble rat-tail tendon collagen. Biochim Biophys Acta 534: 73–81
6. Hamlin CR, Kohn RR, Luschin JH (1975) Apparent accelerated aging of human collagen in diabetes mellitus. Diabetes 24: 902–904
7. Galeski A, Kastelic J, Baer E, Kohn RR (1977) Mechanical and structural changes in rat tail tendon induced by alloxan diabetes or aging. J Biomech 10: 775–782
8. Kilo C, Vogler N, Williamson JR (1972) Muscle capillary basement membrane changes related to aging and to diabetes mellitus. Diabetes 21: 881–905
9. Andreassen TT, Seyer-Hansen K, Bailey AJ (1981) Thermal stability, mechanical properties and reducible cross-links of rat tail tendon in experimental diabetes. Biochim Biophys Acta 67: 313–317
10. Le Pape A, Muh J-P, Bailey AJ (1982) Characterization of N-glycosylated type 1 collagen in streptozotocin-induced diabetes. Biochem J 197: 405–412
11. Herberg L, Coleman DL (1977) Laboratory animals exhibiting obesity and diabetes syndromes. Metab Clin Exp 25: 59–99
12. Bray GA, York DA (1979) Hypothalamic and genetic obesity in experimental animals: an autonomic and endocrine hypothesis. Physiol Rev 59: 719–809
13. Coffey JW, Mattaliano VJJn, Fiedler-Nagy C, Sullivan AC (1978) Collagen composition of the skin from obese and lean Zucker rats. Proc Soc Exp Biol Med 157: 435–439
14. Grant RA (1964) Application of the auto-analyser to connective tissue analysis: J Clin Path 17: 685–691
15. Soden P, Kershaw I (1974) Tensile testing of connective tissues. Med Biol Engineer 12: 510–518
16. Crews EL, Fuge KW, Oscai LB, Holloszy JO, Shank RE (1969)

Weight, food intake and body composition: Effects of exercise and of protein deficiency. Am J Physiol 216: 359–363
17. Allain JC, LeLous M, Bazin S, Bailey AJ, Delaunay A (1978) Isometric tension developed during heating of collagenous tissues. Relationships with collagen cross-linking. Biochim Biophys Acta 533: 147–155
18. Robins SP, Shimokomaki M, Bailey AJ (1973) The chemistry of the collagen cross-links; age related changes in the reducible components of intact bovine collagen fibres. Biochem J 131: 771–780
19. Bailey AJ, Peach CM, Fowler LJ (1970) Chemistry of collagen cross-links; isolation and characterisation of two intermediate intermolecular cross-links in collagen. Biochem J 117: 819–831
20. Robins SP, Bailey AJ (1972) Age-related changes in collagen: The identification of reducible lysine-carbohydrate condensation products. Biochem Biophys Res Commun 48: 76–84
21. Tanzer ML, Fairweather R, Gallop PM (1972) Collagen cross-links: isolation of reduced N_ε-hexosyl-hydroxylysine from borohydride reduced calf skin insoluble collagen. Arch Biochem Biophys 151: 137–141
22. Viidik A (1973) Rheology of skin with special reference to age-related parameters and their possible correlation to structure. In: Robert L, Robert B (eds) Frontiers of matrix biology 1. Aging of connective tissues. S Karger, Basel, pp 157–189
23. Bailey AJ (1968) Intermediate labile intermolecular crosslinks in collagen fibres. Biochim Biophys Acta 160: 447–453
24. Schneir M, Bowersox J, Ramamurthy N, Yavelow J, Murray J, Edlin-Folz E, Golub L (1979) Response of rat connective tissues to streptozotocin-diabetes. Tissue specific effects on collagen metabolism. Biochim Biophys Acta 583: 95–102
25. Goodson WH, Hunt TK (1979) Deficient collagen formation by obese mice in a standard wound model. Am J Surgery 138: 692–694
26. Dubuc PU, Mobley PWm, Mahler RJ (1975) Elevated glucocorticoids in obese-hyperglycaemic mice. Horm Metab Res 7: 102
27. Herberg L, Kley HK (1975) Adrenal function and the effect of a high fat diet on C57Bl/6J and C57Bl/6J-*ob/ob* mice. Horm Metab Res 7: 410–415
28. Cutroneo KR, Rokowski R, Counts DF (1981) Glucocorticoids and collagen synthesis: comparison of in vivo and cell culture studies. Coll Rel Res 1: 557–568

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