ORIGINAL CONTRIBUTIONS

Z. Ernährungswiss. 21, 2–11 (1982)

ISSN 0044-264 X

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Biochemical characterization of the layers of subcutaneous adipose tissue in the pig body

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(Received December 21, 1981)

Introduction

Subcutaneous adipose tissue has been the subject of numerous studies of its morphology, development, and function (1–8). The internal structure is characterized by loose, fibrous connective tissue accompanied by elastic fibres forming three-dimensional chambers. Aggregated adipocytes, fat "lobules", are accommodated in these chambers.

With increasing age and fat content, adipose tissue of the pig becomes separated into two layers (8) by a sheath of flat connective tissue which is most prominent in the back and flanks. The inner (subfascial) layer may be much thicker than the outer one, both storing large amounts of triglycerides.

Some metabolic and cellular parameters have been described (9–14) in an attempt to differentiate these two layers biochemically. However, while having access to an experiment of selection for high and low lipogenic enzymes and fat deposition in the pig (15), we have taken the opportunity to characterize the adipose tissue layers by some fundamental parameters, which are presented in this paper. Deepened insight into fat formation, partially based on these data, will be contained in the subsequent paper[1]).

Materials and methods

Chemicals, buffer substances, and solvents were from Merck AG, Darmstadt, in highest available purity. Fine biochemicals and enzymes were obtained from Boehringer, Mannheim; Serva, Heidelberg; and Sigma, St. Louis, Mo.

Subcutaneous adipose tissue was taken from anaesthetized pigs (120 and 150 days of age, respectively) over the longissimus dorsi muscle; 2–3 g adipose tissue were immediately separated into inner and outer layers, divided into three aliquots

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[1]) Next paper, p. 12

(A, B, C), well stoppered in small bottles, and frozen at the temperature of liquid nitrogen. The analytic scheme was as follows:

A. Water was determined from the frozen state by lyophilization to constant weight. The dried tissue was pulverized at −196 °C and extracted in a Soxhlet apparatus with low-boiling petrol ether (40–60 °C).

One half of the lipid-free residue was analyzed with the Schneider procedure (16) for RNA with orcinol (17) and DNA with diphenylamine (16), using salmon sperm DNA (Serva) and Torula yeast RNA VI (Sigma) for calibration.

Table 1. Analysis of the layers of subcutaneous adipose tissue in the pig (percent of fresh weight).

Substance	Layers		Ratio	P-value
	outer	inner	$\frac{\text{inner}}{\text{outer}}$	
Total lipids	82.8 ± 2.9 (n = 155)	85.2 ± 2.2 (n = 112)	1.03	< 0.001
Lipid-free dry matter	3.2 ± 0.5 (n = 138)	2.5 ± 0.5 (n = 107)	0.78	< 0.001
Water	13.5 ± 2.4 (n = 157)	11.6 ± 1.9 (n = 114)	0.89	< 0.001

Table 2. Analysis of lipid-free residue from the layers of subcutaneous adipose tissue of the pig.

Substance	Dimension	Layers		Ratio	P-value
		outer	inner	$\frac{\text{inner}}{\text{outer}}$	
DNA	mg/100 g fresh	12.2 ± 1.9 (n = 124)	11.0 ± 2.9 (n = 74)	0.90	< 0.001
RNA	mg/100 g fresh	173 ± 46 (n = 125)	166 ± 52 (n = 78)	0.96	n.s.
Total protein	mg/100 g fresh	2420 ± 480 (n = 134)	1660 ± 36 (n = 88)	0.69	< 0.001
Soluble protein	mg/100 g fresh	780 ± 130 (n = 186)	950 ± 170 (n = 176)	1.22	< 0.001
Hydroxy-proline	mg/100 g fresh	205 ± 51 (n = 137)	116 ± 27 (n = 89)	0.57	< 0.001
Hydroxy-proline	g/100 g lipid-free dry matter	6.5 ± 0.7 (n = 137)	5.2 ± 0.7 (n = 89)	0.80	< 0.001
Potassium	mval/kg fresh	8.5 ± 1.7 (n = 100)	8.6 ± 1.3 (n = 53)	1.01	n.s.
Sodium	mval/kg fresh	14.6 ± 3.8 (n = 100)	12.0 ± 3.1 (n = 53)	0.82	< 0.001
K/Na		0.58	0.72		

The second half of the lipid-free residue was hydrolyzed for 24 hrs at 110 °C and N_2 with 6 N HCl in glass ampoules; aliquots were used for the determination of hydroxyproline with Chloramine T and p-dimethylaminobenzaldehyde (18), hydroxy-L-proline (Merck) serving as standard. Remaining aliquots of the hydrolysate were lyophilized and subjected to Kjeldahl analysis of nitrogen (19) for total protein.

B. Sodium and potassium were determined by atomic absorption spectrometry after wet ashing (HNO_3 suprapur; 3.5 hrs; 170 °C) according to l.c. (20).

C. Enzyme activities were measured at 334 nm in the $30 \times 10^3 \cdot g \times 30$ min supernatant of tissue homogenates, freed from floating lipid material. For acetyl-CoA-carboxylase, the extraction buffer was 0.05 M Tris/HCl, 0.1 M KCl, 0.02 M K-citrate pH 7.4; activity determination by following the release of ADP according to l.c. (21, 22). NADPH-forming dehydrogenases were measured from 0.15 M KCl supernatants as above according to l.c. (23), modified according to l.c. (22, 24). Soluble protein was determined with the Lowry procedure (25), using bovine serum albumin for calibration.

Results and discussion

Data on water and total lipid in subcutaneous adipose tissue of the pig are shown in table 1. The handling of adipose tissue needs attention since H_2O tends to decrease by about one fifth within 1 hour at temperatures above the freezing point.

Table 3. Effect of developmental stage of the layers from the subcutaneous adipose tissue of the pig on the tissue composition (data as percent change from 120th to 150th day of life).

Substance Data refer to fresh weight	Concentration on 120th day		Age-dependent change (percent of 120th day)	
	outer	inner	outer	inner
Total lipids (%)	81.3 ± 2.7 (n = 77)	84.0 ± 2.4 (n = 42)	+ 3.4 (n = 78)	+ 3.0 (n = 70)
Lipid-free dry matter (%)	3.4 ± 0.5 (n = 61)	2.6 ± 0.6 (n = 51)	− 11.8 (n = 77)	− 7.7 (n = 56)
Water (%)	14.7 ± 4.4 (n = 78)	12.7 ± 2.0 (n = 44)	− 15.6 (n = 79)	− 17.3 (n = 70)
DNA (mg/100 g)	13.1 ± 1.9 (n = 58)	11.3 ± 4.1 (n = 16)	− 13 (n = 66)	− 2.7 (n = 58)
RNA (mg/100 g)	208 ± 37 (n = 58)	195 ± 64 (n = 18)	− 33 (n = 67)	− 29 (n = 60)
Total protein (mg/100 g)	2440 ± 470 (n = 65)	1810 ± 490 (n = 34)	− 1.2 (n = 69)	− 11.0 (n = 54)
Soluble protein (mg/100 g)	870 ± 120 (n = 96)	1070 ± 60 (n = 89)	− 19.5 (n = 90)	− 22.4 (n = 87)
Hydroxyproline (mg/100 g)	204 ± 48.6 (n = 68)	123 ± 32.7 (n = 32)	+ 1.0 (n = 69)	− 10.6 (n = 57)
Potassium (mval/kg)	9.6 ± 1.6 (n = 38)	9.6 ± 1.1 (n = 16)	− 22.9 (n = 62)	− 20.8 (n = 37)
Sodium (mval/kg)	15.9 ± 3.4 (n = 38)	13.5 ± 3.6 (n = 16)	− 14.5 (n = 62)	− 22.2 (n = 37)

Tables 1 and 2 demonstrate, except for potassium and RNA, highly significant differences in the composition of the two fat layers of total lipid, water, DNA total and soluble protein, collagen, and sodium.

Collagen has been calculated from hydroxyproline values with 13 % OH-proline as the assumed average content (26), thus neglecting the low hydroxyproline contents in elastin (27) and acetylcholine esterase (28), respectively.

Potassium concentrations reflect "active cytoplasm", *i.e.* the small part of the whole cell which is not occupied by deposited fat. The heterogeneous character of adipose tissue, containing connective tissue, vessels and nerves besides adipocytes proper, prevents an accurate calculation of active cytoplasm in fat cells.

Growth and differentiation are of influence on the differential parameters, as seen in table 3. Most concentrations decrease with age, total lipids increase, but, most importantly, the differences between the two layers essentially persist.

There are, in the biochemical parameters of pig adipose tissue, also differences due to the two sexes (table 4). Except for RNA and potassium, sex-specific differences again may be distinguished between outer and inner layers.

Significance			
outer layer $\frac{120\text{th}}{150\text{th}}$ day	inner layer $\frac{120\text{th}}{150\text{th}}$ day	$\frac{\text{outer}}{\text{inner}}$ layer 120th day	$\frac{\text{outer}}{\text{inner}}$ layer 150th day
< 0.001	< 0.001	< 0.001	< 0.001
< 0.001	< 0.05	< 0.001	< 0.001
< 0.001	< 0.001	< 0.01	< 0.001
< 0.001	n.s.	< 0.02	< 0.2 > 0.1
< 0.001	< 0.001	< 0.3 > 0.2	< 0.7 > 0.6
n.s.	< 0.02	< 0.001	< 0.001
< 0.001	< 0.001	< 0.001	< 0.001
n.s.	< 0.05	< 0.001	< 0.001
< 0.001	< 0.2 > 0.1	no difference	< 0.3 > 0.2
< 0.005	< 0.001	< 0.025	< 0.001

Table 4. Sex differences in the composition of the two layers of subcutaneous adipose tissue in the pig (all data refer to fresh weight).

Substance	Female		P-value		Male	
	outer	inner	outer	inner	outer	inner
Total lipids (%)	85.0 ± 2.0 (n = 87)	86.3 ± 2.0 (n = 59)	< 0.001	< 0.001	80.5 ± 1.8 (n = 68)	84.2 ± 2.2 (n = 53)
Lipid-free dry matter (%)	2.8 ± 0.3 (n = 77)	2.2 ± 0.4 (n = 56)	< 0.001	< 0.001	3.6 ± 0.3 (n = 61)	2.8 ± 0.3 (n = 51)
Water (%)	11.8 ± 1.7 (n = 88)	10.7 ± 1.6 (n = 61)	< 0.001	< 0.001	15.3 ± 1.6 (n = 69)	12.6 ± 1.9 (n = 63)
DNA (mg/100 g)	11.1 ± 1.6 (n = 75)	9.9 ± 1.7 (n = 46)	< 0.001	< 0.005	13.3 ± 1.6 (n = 49)	12.0 ± 3.7 (n = 28)
RNA (mg/100 g)	183 ± 62 (n = 76)	160 ± 64 (n = 47)	< 0.05	> 0.3 < 0.4 n.s.	164 ± 28 (n = 49)	173 ± 44 (n = 31)
Total protein (mg/100 g)	2010 ± 70 (n = 71)	1470 ± 220 (n = 49)	< 0.001	< 0.001	2830 ± 300 (n = 63)	1860 ± 400 (n = 39)
Soluble protein (mg/100 g)	760 ± 180 (n = 86)	930 ± 200 (n = 84)	< 0.02	> 0.1 < 0.2	810 ± 70 (n = 100)	970 ± 170 (n = 92)
Hydroxyproline (mg/100 g)	162 ± 12 (n = 71)	100 ± 21 (n = 50)	< 0.001	< 0.001	249 ± 30 (n = 66)	132 ± 24 (n = 39)
Potassium (mval/kg)	8.6 ± 2.1 (n = 58)	8.9 ± 1.7 (n = 30)	< 0.6 n.s.	> 0.1 < 0.2	8.4 ± 1.5 (n = 42)	8.3 ± 0.9 (n = 23)
Sodium (mval/kg)	12.0 ± 1.6 (n = 58)	10.3 ± 1.2 (n = 30)	< 0.001	< 0.001	17.5 ± 2.8 (n = 42)	13.8 ± 3.5 (n = 23)

Table 5. Influence of breeding selection on the composition of the two layers of the subcutaneous adipose tissue in the pig (all data refer to fresh weight, unless stated otherwise).

Substance	"Low-Fat Line" Layer		P-value		"Hight-Fat Line" Layer	
	outer	inner	outer	inner	outer	inner
DNA (mg/100 g)	13.1 ± 1.9 (n = 69)	12.3 ± 3.8 (n = 34)	< 0.001	< 0.001	11.3 ± 1.6 (n = 55)	9.7 ± 0.6 (n = 40)
RNA (mg/100 g)	156 ± 38 (n = 70)	146 ± 37 (n = 38)	< 0.001	< 0.001	190 ± 51 (n = 55)	187 ± 61 (n = 40)
Total protein (mg/100 g)	2560 ± 620 (n = 69)	1690 ± 490 (n = 34)	< 0.005	> 0.5 < 0.6 n.s.	2290 ± 340 (n = 65)	1640 ± 250 (n = 54)
Soluble protein (mg/100 g)	760 ± 100 (n = 78)	1030 ± 120 (n = 72)	> 0.2 < 0.3	< 0.001	740 ± 120 (n = 108)	860 ± 190 (n = 104)
Hydroxyproline (mg/100 g)	222 ± 60 (n = 68)	126 ± 29 (n = 32)	< 0.001	< 0.001	188 ± 42 (n = 69)	106 ± 25 (n = 57)
Hydroxyproline (g/100 g Lipid-free dry matter)	7.0 ± 0.4 (n = 72)	5.8 ± 0.2 (n = 32)	< 0.001	< 0.001	6.1 ± 0.8 (n = 75)	4.6 ± 0.4 (n = 60)
Potassium (mval/kg)	7.5 ± 0.9 (n = 46)	8.9 ± 1.8 (n = 17)	< 0.001	> 0.05 < 0.1 n.s.	9.5 ± 1.8 (n = 54)	8.3 ± 0.8 (n = 36)
Sodium (mval/kg)	15.4 ± 4.9 (n = 46)	13.3 ± 3.8 (n = 17)	< 0.05	< 0.005	13.8 ± 2.9 (n = 54)	10.8 ± 1.8 (n = 36)

In a breeding experiment for low or high fat content in pigs, with enzymological and gross morphometric parameters for selection, again the differential characters of the two layers of adipose tissue are essen-

Table 6. Activities of some enzymes concerned with the biosynthesis of fatty acids in the layers of the subcutaneous adipose tissue in the pig (all data as milli-units/mg soluble protein).

	outer	P-value	inner
Acetyl-CoA carboxylase			
all animals	73 ± 52 (n = 186)	< 0.001	93 ± 53 (n = 176)
"high-fat line"	99 ± 65 (n = 108)	n.s.	107 ± 74 (n = 104)
"low-fat line"	47 ± 15 (n = 78)	< 0.001	78 ± 23 (n = 72)
120th day of life	92 ± 63 (n = 96)	no difference	92 ± 66 (n = 86)
150th day of life	74 ± 54 (n = 88)	< 0.02	93 ± 47 (n = 83)
female	101 ± 57 (n = 86)	< 0.001	130 ± 51 (n = 84)
male	45 ± 32 (n = 100)	< 0.005	56 ± 18 (n = 92)
Malic enzyme			
all animals	1120 ± 528 (n = 177)	< 0.001	1298 ± 452 (n = 169)
"high-fat line"	1550 ± 316 (n = 99)	< 0.02	1653 ± 289 (n = 97)
"low-fat line"	690 ± 241 (n = 78)	< 0.001	943 ± 239 (n = 72)
120th day of life	1295 ± 514 (n = 87)	> 0.1 < 0.2	1410 ± 400 (n = 81)
150th day of life	1178 ± 511 (n = 88)	n.s.	1185 ± 532 (n = 86)
female	1477 ± 546 (n = 84)	n.s.	1435 ± 545 (n = 82)
male	983 ± 459 (n = 93)	< 0.005	1160 ± 359 (n = 87)
Glucose-6-phosphate dehydrogenase			
all animals	276 ± 183 (n = 177)	near < 0.05 tendency	293 ± 162 (n = 171)
"high-fat line"	378 ± 210 (n = 99)	n.s.	390 ± 185 (n = 99)
"low-fat line"	175 ± 83 (n = 78)	< 0.001	215 ± 60 (n = 72)
120th day of life	398 ± 183 (n = 87)	n.s.	383 ± 189 (n = 83)
150th day of life	155 ± 76 (n = 88)	< 0.001	203 ± 61 (n = 86)
female	313 ± 207 (n = 84)	n.s.	330 ± 186 (n = 82)
male	240 ± 179 (n = 93)	n.s.	255 ± 152 (n = 89)
6-Phosphogluconate dehydrogenase			
all animals	212 ± 122 (n = 177)	n.s.	205 ± 106 (n = 171)
"high-fat line"	255 ± 115 (n = 99)	> 0.1 < 0.2	278 ± 108 (n = 99)
"low-fat line"	145 ± 60 (n = 78)	> 0.1 < 0.2	133 ± 21 (n = 72)
120th day of life	270 ± 99 (n = 87)	> 0.2 < 0.3	250 ± 138 (n = 83)
150th day of life	130 ± 43 (n = 88)	< 0.001	160 ± 38 (n = 86)
female	225 ± 115 (n = 84)	no difference	225 ± 125 (n = 82)
male	157 ± 100 (n = 93)	> 0.05 < 0.1 tendency	185 ± 97 (n = 89)
Isocitrate dehydrogenase			
all animals	195 ± 120 (n = 177)	> 0.1 < 0.2	178 ± 115 (n = 171)
"high-fat line"	238 ± 143 (n = 99)	n.s.	250 ± 128 (n = 99)
"low-fat line"	153 ± 92 (n = 78)	< 0.001	105 ± 25 (n = 72)
120th day of life	295 ± 77 (n = 87)	< 0.005	240 ± 140 (n = 83)
150th day of life	95 ± 33 (n = 88)	< 0.001	120 ± 23 (n = 86)
female	208 ± 126 (n = 84)	> 0.3 < 0.4	190 ± 128 (n = 82)
male	183 ± 132 (n = 93)	> 0.3 < 0.4	165 ± 119 (n = 89)

tially preserved (table 5). One would conclude then that the differentiation of these two layers is influenced by growth, sex, and genetic constitution.

When enzyme activities concerned with some steps of fatty-acid biosynthesis are measured in the two layers (table 6), there are rather profound and persistent differences in acetyl-CoA-carboxylase, and it is demonstrated in table 6 that growth, sex, and breeding experiments express themselves also in enzymatic data of adipose tissue. Besides the rate-limiting step of fatty-acid synthesis, catalyzed by acetyl-CoA-carboxylase, the four major NADPH-producing dehydrogenases are presented in table 6; they follow more or less closely the distribution pattern of acetyl-CoA-carboxylase.

In most cases, the inner layer of subcutaneous adipose tissue contains higher activities than the outer layer. Although the five enzymes listed in table 6 may behave like a "block of lipogenic enzymes" (next paper), they may still be distinguished by the extent of alteration caused by growth, sex, *etc.*

The female pig of 100 kg carries the following activities of acetyl-CoA-carboxylase in the subcutaneous adipose tissue:

inner layer 99 ± 65 ($n = 93$) mU/mg soluble protein,

outer layer 69 ± 46 ($n = 93$) mU/mg soluble protein,

with $p < 0.001$.

In conclusion, peculiarities of adipose tissue composition are apparent in the anatomical layers which obviously suggest functional consequences (see next paper). In addition, sodium concentrations are higher than ever could be expected since the Na^+ measured is distributed in the low-sodium cytoplasm (with really small volumes in adipocytes) and in the extracellular space (of unknown volume). Based on experience in other tissues like e.g. liver and skin (29), one has to assume that part of the sodium in adipose tissue cannot exist as free ions, but must be bound in such a manner that it does not contribute to membrane potentials and osmotic pressures, just as it is the case for bone and skin (30).

In the light of factors influencing the differences between adipose tissue layers, one might ask whether nutritive factors contribute or not. First indications of a positive answer will be found in the next paper, but, generally speaking, nutrition modifies adipose tissues in so many aspects that a thorough study of this problem requires an effort of its own.

Acknowledgment

The authors are grateful to Profs. Fewson and Menke for discussions and advice, and to Dr. Rogdakis for making possible the bioptic sampling of tissue. The work was supported by DFG–SFB 142–TPB2.

Summary

During the formation of two layers of adipose tissue in the pig's body, starting from the 80th day after birth, samples were obtained by biopsy and analyzed for gross constituents and enzymes concerned with fatty-acid biosynthesis. These two layers differ in total lipid and water content and demonstrate more subtle differences amongst DNA, protein, collagen, and sodium concentrations when comparisons are made in regard to age, sex, and breeding selection for low-fat animals.

Acetyl-CoA carboxylase, malic enzyme and glucose-6-phosphate dehydrogenase are more active in the inner layer, while 6-phosphogluconate and isocitrate dehydrogenases are distinguishable in the two layers of adipose tissue as well if age, sex,

and breeding lines are taken into consideration. The data form the basis for a more detailed study of lipogenic potentials in adipose tissue (next paper).

Zusammenfassung

Während der Ausbildung von Schichten im Fettgewebe am Schweinerumpf ca. ab dem 80. Tag nach Geburt wurden bioptisch Gewebsproben gewonnen und analysiert auf Hauptbestandteile und auf Enzyme, die bei der Fettsäuren-Biosynthese mitwirken. Die beiden Schichten unterscheiden sich im Fett- und Wassergehalt und zeigen auch bei DNS-, Protein-, Kollagen- und Natrium-Konzentrationen feinere Differenzen, wenn verschiedene Lebensalter, Geschlechter und Zuchtlinien auf fettarme Tiere verglichen werden.

Acetyl-CoA-Carboxylase, Malic enzyme und Glucose-6-phosphat-Dehydrogenase sind in der inneren Schicht stärker aktiv; 6-Phosphogluconat-Dehydrogenase und Isocitrat-Dehydrogenase sind ebenfalls in den beiden Schichten unterscheidbar, wenn Alter, Geschlecht und Zuchtlinien in die Betrachtung einbezogen werden. Die Ergebnisse bilden die Grundlage für eine ins einzelne gehende Untersuchung des lipogenen Potentials im Fettgewebe (nächste Arbeit).

Key words: adipose tissue, acetyl-CoA-carboxylase, NADPH-forming dehydrogenases, lipid formation

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