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Changes in weight and compositions of major membrane components of human brain during the span of adult human life of Swedes

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Abstract Brain weight, total solids, protein, and major lipids have been determined in 83 female and 101 male brains from subjects 20–100 years of age. The brain weight began to diminish at 20 years of age. The brain weight at 20 years for females: 1,368 ± 26 and for males 1,632 ± 27 g diminished at 100 years for females to 1,100 ± 25 and for males to 1,266 ± 25 g, a decrease of 20% for female and 22% for male brains. The decrease in dry solids was larger during the same period, 36% for females and males. Proteins decreased by 39% in females and 37% in males. Phospholipids decreased by 42% in females and 43% in males, cholesterol by 47% and 53%, cerebroside by 46% and 58%, sulfatide by 46% and 49% and gangliosides by 28% and 30%, respectively. There is, thus, a significantly larger loss of myelin lipids than of gangliosides – the biochemical marker for neuronal membranes. The loss of myelin lipids was particularly large in female brain after 70 years of age, while the loss in male brain was linear as early as from 20 years of age.

Key words Brain weight · Ageing · Myelin lipids · Gangliosides · Proteins

Introduction

In a recent study of membrane lipids in the ageing human brain [1], we found that the concentration of dry weight and major lipids diminished continuously in frontal and temporal cortices and white matter from 20 years of age. Gangliosides were an exception to the rule and reached their highest values in the frontal and temporal cortices between 40 and 50 years of age. The percentage losses in the concentrations of major lipids between 20 and 100 years of age were 15–20% in the frontal and temporal cortices and 30–35% in white matter. The study was performed on brains from individuals who, until death, had normal social life with no history of neurological or psychiatric disease, and who died suddenly and unexpectedly. It was remarkable that the large loss of membrane lipids did not give any overt sign of reduced function, particularly when one takes into account the marked reduction of brain weight that also occurred during the same period. To get a more complete picture of the losses of brain membrane components, we decided to examine the changes during ageing in the whole brain. We used the same standard conditions for the collection of brains as in the previous two publications [1, 2].

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Materials and methods

Tissue

Brains were obtained from individuals 20–100 years of age, 83 females and 101 males, who died suddenly and unexpectedly. The numbers of females and males assigned to four age groups are given in Table 1. The individuals from 20–65 years of age had all been in full exercise of a profession. The majority were killed in accidents, with no damage to the head. Among the 22 females of 20–40 years of age five of them were small women who died by drowning. About 50% of the men between 50 and 69 years died of acute myocardial infarction. The individuals between 60 and 100 years of age had all been living in their own homes with the oldest persons only receiving some assistance from family members,

Table 1 Number and age of female and male brains collected

	20–39 years	40–59 years	60–79 years	80–100 years
Females	22	20	12	29
Males	22	26	35	18

and/or the social service. The police reports, based on interviews with family members and/or neighbors and social welfare assistants, ruled out neurological and psychiatric symptoms or other serious chronic disorders. Approximately 50% of the persons over 90 years of age had slight symptoms of dementia and received their main daily meal via the social services. The autopsies were all performed on bodies which had been refrigerated, usually within 48–72 h and never more than 72 h after death. The brain stem was divided from the spinal cord just below the decussation of the pyramids, approximately 2 cm below the lower level of the pons. The brain was dissected, the main trunks of intracranial arteries removed, but the leptomeninges left intact. The brain was divided by a midline saggital section into two halves, which were weighed immediately after the cerebrospinal fluid had been emptied and the walls wiped with cellulose fluff. The weighing was performed on a magnetic force compensation balance (Mettler P 1200) with an accuracy of ± 0.1 g, which was regularly calibrated during the study. The right half of the brain was used for the biochemical analyses of whole brain tissue, while the left half was used for microscopic and biochemical examinations of a large number of areas [1]. The right half of the brain of 17 females was divided with a section between diencephalon and mesencephalon and two portions were obtained: forebrain and cerebellum + brain stem – diencephalon. No brains revealing pathological changes on macroscopic or microscopic examination were accepted for the study. The biochemical examination of frontal and temporal lobes also had to show values within two standard deviations of the mean found in the previous study [1] before the brain specimen was accepted for the present study.

Homogenization and dry weight determination

The right brain half was homogenized in a Waring blender under constant cooling at +4°C. The homogenizer was run for 10 min. Duplicate samples of approximately 100 mg were transferred to small tubes, weighed and dried by lyophilization. The tissue was then dried to constant weight in a desiccator over granular phosphorous pentoxide, weighed, and dry solids were calculated.

Determination of protein and lipids

Duplicate samples of approximately 100 mg were suspended in exactly 0.7 ml of water and ultrasonicated for 5 min. The suspended tissue homogenate was added to 3.0 ml chloroform/methanol 1:2, to give a final ratio chloroform/methanol/water 4:8:3 (by volume) [3]. The supernatant was collected by centrifugation for 10 min at 1,000 g, the pellet reextracted with the same solvent mixture, and the supernatant again obtained by centrifugation. The two combined supernatants were evaporated and then dissolved into 5 ml chloroform/methanol/water 60:30:4.5 (by volume). The pellet was dried in a desiccator over phosphorus pentoxide after removal of the organic solvent in a vacuum desiccator and weighed. The lipid content was determined by subtracting the weight of the lipid-extracted pellet from its weight as dry solid, assuming that the pellet consisted of protein only (the weight of nucleic acid and polysaccharides was considered to be negligible). The pellet was then used for determination of protein-bound sialic acid [4].

Half of the extracted lipids (2.5 ml) was put into a 0.5-g column of Sephadex G-25. When the extract has passed through, it was eluted with 2.5 ml chloroform/methanol/water (60:30:4.5) and 25 ml chloroform/methanol (2:1). The three eluates were collected in a small round flask and evaporated to dryness. The lipids were finally dissolved in exactly 5.0 ml chloroform/methanol/water (60:30:4.5). This lipid extract was used for the assay of cholesterol, phospholipids, cerebroside and sulfatide. The second half of the extracted lipids (2.5 ml)was mixed with 0.5 ml chloroform and applied to a 1-g silica gel column packed in chloroform. The column was eluted with 8 ml chloroform/methanol/water (65:25:4). The ganglioside fraction was subsequently eluted with 10 ml chloroform/methanol/water (3:6:2). This latter fraction was used for the determination of total gangliosides and ganglioside pattern.

Assay methods

Cholesterol was assayed with a ferric chloride method [5], phospholipids with a modified Fiske-Subbarow method [6], and cerebroside and sulfatide were assayed by quantitative densitometry after thin-layer chromotography (TLC) separation [7]. Ganglioside sialic acid was assayed by the resorcinol method [8], and the ganglioside pattern was determined using densitometry at 620 nm after separation of the gangliosides on HPTLC plates developed with chloroform/methanol/0.25% aqueous KCl (50:40:10) and visualization using the resorcinol reagent.

Statistics

The precision of homogenization and quantitative determinations was calculated from determinations of 50 duplicate samples. The coefficient of variation was as follows: dry weight 1.2%, protein-bound sialic acid 2.7%, lipid-bound sialic acid 2.1%, phospholipids 1.8%, cholesterol 1.9%, cerebroside 1.9% and sulfatide 2.8%. Analysis of variance followed by multiple comparisons, using the Bonferoni criterion, was performed when means of adjacent groups were compared. Estimates of mean predicted values and associated standard error of mean were calculated, by fitting a non-linear regression model to data. The fitted model was defined as $Y = a*10^{-b*X}$, where a stands for the intercept and b the elimination rate constant. Y and X correspond to the dependent and the independent variables, respectively. The NLIN* procedure in the statistical package SAS version 6.11 was applied in these analyses. Taking the 10-logarithm of the Y values, a linear model was generated from which P values for the slope were obtained. The estimating equation for the linear model that was applied on log-transformed Y values was: $Log(Y) = a-b*X$, where b stands for the slope for the predicted line. EXCEL 5.0 was used to make the graphs.

Results

Several different mathematical formulas were tested to examine which formula best fitted the values found. We chose to express the values for brain weight and the other parameters in logarithmic terms, and the age in linear terms, but most parameters showed an almost linear relationship between parameter and age. The values in Table 2 are expressed as the predicted values, means and standard error of means at 20, 40, 60, 80 and 100 years of age. From these values it is simple to estimate the predicted value for all ages between 20–100 years of age.

Brain weight

Because of the limited number of brains and the suspicion that subjects of same age groups deviated from the mean brain weight of the age, we decided to give the values observed in one table (Table 3) and the predicted values for a certain age as calculated from all values found with the semilogarithmic method as described in Materials and methods (Table 2). The predicted values coincided with the observed values for the males between 20 and 90 years, but for the females the predicted values were lower than the observed values between 20 and 80 years and thereafter higher. It was, thus, evident that the semilogarithmic method was not valid for the whole age period, but there

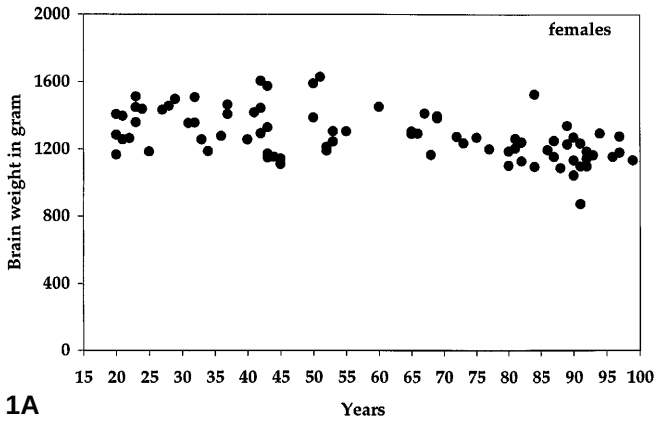
Table 2 Changes in weight and content of proteins and major lipids of human brain with age: predicted values after statistical treatment of whole data (*f* females, *m* males, *M* mean, *SEM* standard error of mean)

Biochemical constituents	Sex	20 years		40 years		60 years		80 years		100 years		Percentage loss 20–100 years
		M	SEM	M	SEM	M	SEM	M	SEM	M	SEM	
Brain weight (g)	f	1,368	26	1,336	17	1,304	14	1,226	18	1,100	25	20
	m	1,632	27	1,525	16	1,425	13	1,335	18	1,266	25	22
Dry solids (g)	f	301	10	287	7	271	5	245	7	192	9	36
	m	369	11	332	6	299	6	269	8	235	11	36
Proteins (g)	f	132	4	121	3	115	2	101	3	80.6	3.6	39
	m	155	5	140	3	126	3	110	4	96.5	5.0	37
Phospolipids (mmol)	f	102	3	95.4	2.1	89.1	2.0	77.2	2.3	59.6	3.0	42
	m	124	4	110	2	97.9	2.0	84.2	2.9	70.6	3.8	43
Cholesterol (mmol)	f	90.4	3.7	86.5	2.3	80.1	2.0	67.3	2.5	47.8	3.2	47
	m	113	4	98.6	2.2	85.4	2.0	75.3	2.8	64.0	3.6	53
Cerebroside (mmol)	f	32.3	1.1	31.7	0.3	29.4	0.1	24.1	1.0	17.3	1.3	46
	m	44.9	1.3	38.9	0.3	33.2	0.8	26.2	1.0	18.9	1.3	58
Sulfatide (mmol)	f	11.2	0.6	10.2	0.3	9.3	0.3	7.6	0.4	5.2	0.5	54
	m	11.7	0.4	11.3	0.3	10.0	0.3	7.9	0.4	5.7	0.6	51
Ganglioside (mmol NeuAc)	f	3.81	0.12	3.69	0.08	3.64	0.07	3.26	0.08	2.76	0.11	28
	m	4.66	0.14	4.26	0.08	3.88	0.07	3.49	0.10	3.27	0.14	30
Sialoglycoprotein (mmol NeuAc)	f	1.20	0.03	1.14	0.02	1.08	0.02	1.03	0.02	0.97	0.03	19
	m	1.57	0.05	1.38	0.03	1.27	0.02	1.16	0.04	1.06	0.05	30

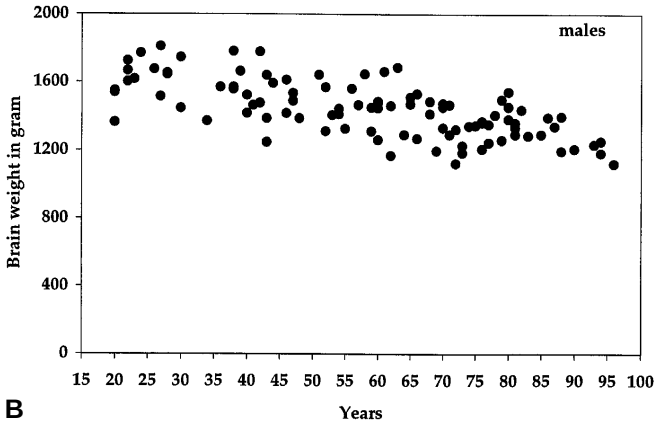
Table 3 Changes in the concentration of dry solids, protein and major lipids of human brain with age, mean values of each age group (*f* females, *m* males, *M* mean, *SD* standard deviation)

Biochemical constituents	Sex	20–39 years		40–59 years		60–79 years		80–100 years	
		M	SD	M	SD	M	SD	M	SD
Brain weight (kg)	f	1,365	109	1,321	168	1,303	88	1,177	112
	m	1,595	139	1,479	125	1,372	135	1,314	108
Dry solids (kg)	f	216	5	215	14	204	12	185	9
	m	220	17	214	15	201	10	193	6
Proteins (kg)	f	94	5	89	10	85	6	78	7
	m	90	6	92	8	86	5	80	4
Phospolipids (mmol/kg)	f	73	2	70	6	67	4	59	3
	m	74	5	70	5	66	5	61	3
Cholesterol (mmol/kg)	f	65	3	63	8	59	4	49	5
	m	68	5	61	5	58	5	54	2
Cerebroside (mmol/kg)	f	24	2	24	2	22	2	18	2
	m	27	2	24	2	22	2	18	2
Sulfatide (mmol/kg)	f	8.0	1.6	7.2	1.0	7.4	1.4	5.6	1.1
	m	7.2	0.9	7.6	0.9	6.7	0.8	5.5	0.3
Ganglioside (mmol NeuAc/kg)	f	2.77	0.14	2.66	0.14	2.76	0.12	2.58	0.18
	m	2.83	0.20	2.76	0.11	2.66	0.18	2.60	0.11
Sialoglycoprotein (mmol NeuAc/kg)	f	0.86	0.08	0.84	0.04	0.83	0.03	0.82	0.11
	m	0.91	0.08	0.92	0.09	0.82	0.08	0.86	0.07

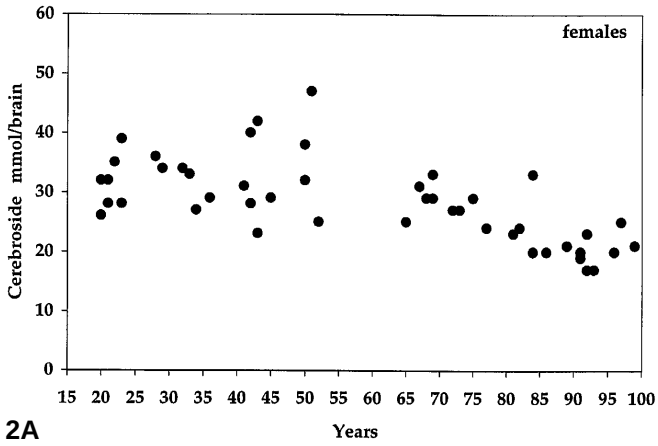
was an inflection point around 70 years of age (Fig. 1A). When we used one equation for 20–70 years and a second for 71–100 years of age, the predicted and the observed values also coincided for females. The brain weight was reduced by 20% for females and by 22% for males between 20 and 100 years, but the difference was larger between female and male brains between 20–29 years and 70–79 years (see Table 6). During this period the weight loss was only 7.6% for females but 15.9% for male brains.



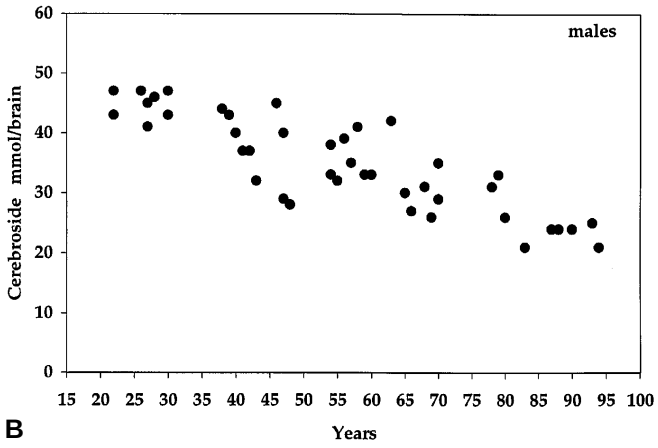
1A



B



2A



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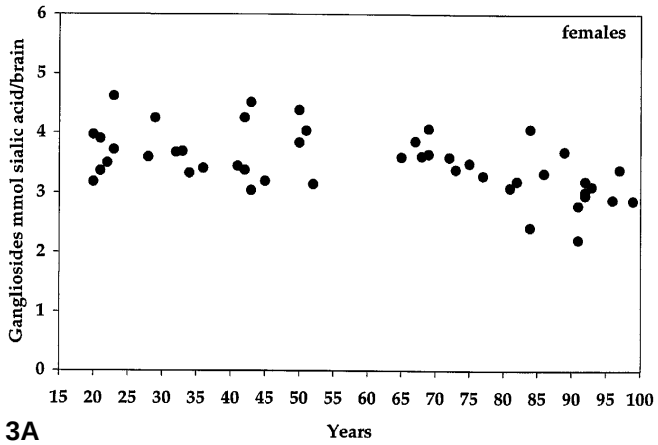
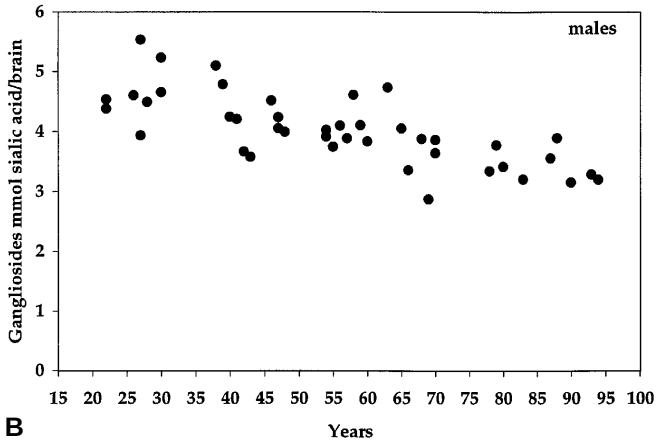


3A



B

Fig. 1 Weight of brain by age in females (**A**) and males (**B**)

Fig. 2 Content of cerebroside by age in females (**A**) and males (**B**)

Fig. 3 Content of gangliosides (mmol NeuAc) by age in females (**A**) and males (**B**)

Content of biochemical constituents

Dry solids and proteins

The loss of dry solids was significantly larger than the diminution in brain weight, 36% for female and male brains between 20 and 100 years of age, indicataing that a significant portion of solid brain constituents had been replaced with water – the water concentration increased from 78% at 20 years of age to 82% at 100 years. The loss of proteins was of about the same magnitude as that of total solids.

Major lipids

In female brains, the phospholipid diminution between 20 and 100 years was 42% and for the more myelin-characteristic lipids cholesterol and cerebroside 47% and 46%, respectively (Table 2). In male brains, the loss of phos-

Table 4 Concentration of biochemical constituents in forebrain and cerebellum + brain stem – diencephalon in brains of 17 females aged 20–60 years (*n.s.* not significant)

Biochemical constituents	Forebrain		Cerebellum		Difference
	M	SD	M	SD	
Weight (g)	1144	118	176	20	$P < 0.001$
Dry solids (g/kg)	213	11	204	14	$P < 0.05$
Proteins (g/kg)	90	9	94	9	n.s.
Phospholipids (mmol/kg)	72	5	64	5	$P < 0.0001$
Cholesterol (mmol/kg)	63	5	53	5	$P < 0.0001$
Cerebroside (mmol/kg)	24	2	20	2	$P < 0.0001$
Sulfatide (mmol/kg)	8.3	1.4	6.8	1.0	$P < 0.001$
Gangliosides (mmol NeuAc/kg)	2.75	0.17	2.43	0.21	$P < 0.0001$
Sialoglycoproteins (mmol NeuAc/kg)	0.88	0.007	0.71	0.008	$P < 0.0001$

pholipids and cholesterol was 43%, and of cerebroside 58%. The reduction of the sulfuric acid ester of cerebroside, sulfatide was, however, similar between female and male brain of 54% and 51%, respectively.

Gangliosides and sialoglycoprotein

The reduction of gangliosides during the period studied was only 28% in female and 30% in male brains and almost the whole reduction in female brain occurred after 70 years of age. The loss of sialoglycoproteins was even lower than that of gangliosides, in female brain only 20%, while it was the same as of gangliosides in male brain 30%.

Concentration of biochemical constituents

The reduction in the concentration of lipids enriched in myelin, cholesterol and cerebroside varied between 22% and 31%, the reduction of cholesterol being largest in female brain, while cerebroside was most reduced in male brain. The diminution of ganglioside concentration was significantly less, only 6% and 8% in female and male brain, and the concentration in female brain was equally high at 70 years as at 20 years.

Forebrain and cerebellum

In 17 cases the forebrain – cerebrum and diencephalon – was dissected from the remaining brain stem and cerebellum and the two portions were homogenized and assayed separately. The cerebellar portion was 13% of the whole brain. The concentration of major lipids was significantly higher in cerebrum than in cerebellum (Table 4), while the concentration of proteins did not differ. Nevertheless, the lower lipid values in cerebellum had only a marginal effect on the values in the whole brain. We did not see any differences in the concentration of lipids in cerebellum and brain stem with age in this limited material.

Ganglioside pattern

The ganglioside pattern underwent small but significant changes with age. The proportion of gangliosides of the a-series, GM1 and GD1a, diminished and that of b-series increased (Table 5). The proportion of GD1b increased throughout the entire period studied while the proportion of GT1b increased less and reached its maximum between 60 and 79 years. The ganglioside pattern of cerebellum compared to that of forebrain had significantly larger proportions of b-series gangliosides, particularly GT1b and GQ1b, while the proportions of GM1 and GD1a were markedly smaller (Table 4). The influence of cerebellum, and brain stem on the ganglioside pattern of the whole brain was small but significant (Tables 4, 5).

Discussion

This study is concerned with the composition of the major biochemical constituents of brain membranes and their changes throughout the span of human life. There is very little published data on the chemical composition of the whole human brain. We are only aware of two comprehensive studies, by Bürger [9] and Rouser and Yamamoto [10, 11]. In a recent study of membrane lipids in the ageing human brain, we demonstrated large individual variations in the hydration of the brain [1], while the differences in the composition of the brain dry solids were small. For this reason it is more appropriate to express the results as content per brain, as in this study, rather than in

Table 5 Ganglioside pattern of female and male brain with age. Several other gangliosides were determined, GM3, GM2, GD2, GT1a, but as they did not show any significant changes with age they are not reported in the table, although they are included in the 100% figure (*f* females, *m* males, *M* mean, *SD* standard deviation)

Ganglioside constituents	20–39 years		40–59 years		60–79 years		80–100 years	
	M	SD	M	SD	M	SD	M	SD
GM4	4.0	0.8	4.3	0.9	3.9	0.7	4.2	0.9
GM1	16.2	1.8	15.4	1.3	14.3	0.9	13.2	1.4
GD3	3.9	0.9	4.2	0.9	4.1	1.0	4.9	1.0
GD1a	22.3	1.6	20.3	1.5	19.3	2.0	18.4	2.2
GD1b	20.4	2.1	22.4	1.0	23.5	1.6	24.0	1.9
GT1b	23.1	2.0	24.3	1.2	25.1	1.8	24.7	1.9
GQ1b	4.0	1.1	4.4	0.9	4.2	0.4	4.3	0.6

percentage of fresh weight as given by Bürger [9] and Rouser and Yamamoto [10, 11]. However, we obtained our results by examining a portion of the whole brain homogenate, and our primary results were calculated in concentration of fresh weight, as in the previous studies, before we multiplied the values by the brain weight. The results for the concentration of the lipids are also given to allow comparison with the previous published values (Table 3).

The crucial problem in a study of the changes in biochemical constituents of the brain during ageing is the selection of the brain material. To eliminate subjects with chronic diseases or the influence of agonal changes, we decided to collect brains from subjects with no history of ongoing disease or previous abuse of alcohol or drugs and who died suddenly. For this reason, only cadavers referred to the Department of Forensic Medicine were included because the records of the subjects referred to the Department were carefully drawn up by police and social institutions. Body fluids from the cadavers were all examined for signs of alcohol and drugs. It was a difficult task to obtain cadavers that complied with all the inclusion criteria. Subjects killed in traffic accidents or accidents at work almost always had damage to the brain, subjects who committed suicide often did so with drugs, car exhaust or a gunshot fired at the head. We have collected brains for more than 5 years, but we have still not obtained an optimal number of brains from females of 20–70 years of age. To eliminate bias at the collection, we decided to include every subject who fulfilled our inclusion criteria. By chance we might have an overrepresentation of small female subjects 20–39 years of age and of tall, athletic men of the same age group.

Because of the limited number of brains and the suspicion that subjects of same age groups deviated from the mean brain weight of that age, we decided not to give the values observed but rather the predicted value for a certain age, calculated from all the values found, using a semilogarithmic method as described in Materials and methods and also used by Rouser and Yamamoto [10, 11]. The predicted values coincided with the observed values for the males, but the predicted values for the brain weight and the biochemical constituents of female brain were lower than the values found between 20 and 80 years and higher than predicted after 80. It was thus evident that the semilogarithmic linear equation was not valid and that there was an inflection point at approximately 70 years, as can be seen in Fig. 1b. We have, therefore, used one equation for 20–70 years and a different one for 70–100 years of age. Our present values for the brain weight in male subjects 20–29 years of age are higher than any previously recorded values. Skullerud [12] found higher brain weight for Norwegian males aged 45–54 years than ours, but she claims that there was no decrease in brain weights in her material before 50 years of age. However, she found a rapid decrease in brain weight of 11% between 45–54 and 70–79 years compared with a decrease of 7% in our study, so her values and ours are the same at 79 years of age. In contrast Skullerud [12] found slightly lower values for the brain weight of Norwegian females 45–54 years of age than we, and the decrease at 70–79 years was also slightly greater. The discrepancies between our two studies reflect the difficulties in obtaining a sufficiently large representative brain material for a single laboratory. Dekaban and Sadowsky [13] tried to solve the problem by recording the brain weights obtained by five laboratories. They succeeded in obtaining the weights of more than 4,000 subjects, but there is no report about how long the collection period was or how many of the patients had suffered from debilitating diseases before death. They found that the decrease in brain weight began in females from 20 years of age but in males not until 41–50 years of age, and that the decrease in both females and males was 7% between 25 and 75 years but 15% for females and 11% for males up to 86+. This also suggests that even a large sample of brains gives uncertain values for the brain weight at a certain age. Studies of brain weights in Germany by Handmann in 1906, by Rössle and Roulet in 1932 and by Bürger in 1957 have been summarized by Bürger [9]. Their data about the starting point and the extent of decrease of brain weight are in agreement with ours. The brain weight was found to be highest between 20–30 years of age in females and males and the reduction of weight was greatest between 81 and 90 years, the highest age recorded. Skullerud [12] demonstrated that the volume of the lateral ventricles was 1.7% of the brain weight at 45–54 years of age and 3.0% at 70–79 years. This increase of volume accelerates after 80 years of age. In contrast to Skullerud and ourselves, Pakkenberg and Voigt [14] and Dekaban and Sadowsky [13] weighed the brain with the cerebral ventricles unopened. If they had removed the cerebrospinal fluid as we did their values would have been lower and their reduction with ageing greater. It is only when the brain is drained of cerebrospinal fluid that we can get a true picture of the loss of brain tissue with age.

Our number of brains was too small to allow any regression analyses with variables other than age and sex, e.g. height and body weight. Several studies [12, 14–16] have shown a gain in weight of 2–4 g/cm of height and no significant difference for weight, which means that these two variables are of minor importance compared with age and sex. Nevertheless, in our material five women 20–29 years were an average of 11 cm below the mean length for their age. Using Skullerud‘s figure their brain weights can be considered to have been less than that of the standard woman for the age. These five women might have slightly reduced the brain weight figures and biochemical constituents for females 20–29 years of age in our material.

The highest brain weights have been found in the most recent studies from Scandinavian countries (Table 6). This finding suggests that secular changes associated with high educational and nutritional levels might have played an important role for the size of the brain. These factors might be pronounced in Scandinavia with its homogenous population and high, even social standard. In our own study higher brain weights were found than in any previous study, particularly in the youngest age group. The

Table 6 Means of brain weights in grams during two 10-year periods (20–30 years and 70–80 years of age) in 12 different studies

	Males			Females		
	Age 20–30 years	Age 70–80 years	% Change	Age 20–30 years	Age 70–80 years	% Change
Marshall (1892)	1,343	1,290	–3.9	1,239	1,170	–5.6
Handmann (1906)	1,392	1,282	–7.9	1,252	1,175	–6.2
Chernyshev (1911)[a]	1,383	1,308	–5.5	1,244	1.175	–5.6
Ellis (1920–21)	1,360	1,292	–5.0	1,241	1,172	–5.6
Mühlman (1927)[b]	1,402	1,290	–8.0	1,264	1,145	–9.5
Rössle and Roulet (1932)	1,395	1,266	–9.2	1,233	1,150	–6.7
Bürger (1957)	1,336	1,176	–12.0	1,240	1,060	–14.5
Pakkenberg and Voigt (1964)[a]	1,526	1,395	–8.0	1,366	1,222	–10.6
Chrzanowska and Beben (1973)	1,463	1,362	–7.0	1,312	1,219	–7.1
Dekaban and Sadowsky (1978)	1,449	1,344	–7.3	1,309	1,213	–7.4
Skullerud (1985)	1,527[b]	1,361	–10.9	1,292[b]	1,223	–5.3
Svennerholm et al. (present study)	1,607	1,352	–15.9	1,360	1,257	–7.6

[a] Value recalculated by the present authors
[b] 45–54 years of age instead of 20–30

subjects included in the age group 20–39 years of age all grew up after World War II, when Sweden has reached its highest educational and nutritional standard. The differences in brain size of our subjects and subjects from previous studies were smaller in subjects aged 70 years and over, even taking into account the weight of cerebrospinal fluid in Pakkenberg and Voigt [14] and Dekaban and Sadowsky [13] studies. Subjects of this age or older grew up during a period when many families could not afford to give their children good nutrition or education. Brain weights have not only increased in Scandinavia; Table 6 also shows a generalized increase since 1900. The hypothesis of a considerably accelerated increase in the weight of the brain in the last 70 years was first formulated by Röthig in 1974 [17], although he had no data to support it. With the results presented by Skullerud and ourselves, it might be more relevant to formulate Röthig's hypothesis as: "a considerable increase in the weight of human brain has occurred over the last 90 years".

It is clear from Table 6 that the size of our female and male brains is close to findings from other recent studies. This suggests that the content of biochemical constituents and their changes with age has been determined on relevant brain material. This has been important for us to confirm, because we have performed a pioneering study which will be difficult to repeat in the future. Our study has shown that both female and male brain decreased 36% in dry solids between the age of 20 and 100 years, i.e., that the brain lost more than 1/3 of its building materials used mainly for membrane formation. There are two main sources of brain membranes – neuronal membrane and myelin. Human myelin consists of 30% protein and 70% lipids [18], the most characteristic of which is cerebroside which was suggested earlier to be the optimum lipid marker for myelin [19]. However, cholesterol and phospholipids also occur in much higher concentrations in myelin than in neuronal membranes. Gangliosides have been used as a marker for neuronal membrane [20] for as long as cerebroside has been used for myelin. Our study has shown that myelin lipids were lost early and that the loss exceeded 40% at 100 years of age (Table 2). Particularly in male brain there is a considerable loss of cerebroside, while the loss of cholesterol is equally large as that of cerebroside in female brain. There are also other differences between female and male brains. The content of myelin lipids, particularly cerebroside, only shows a moderately slow decline before 70 years of age in female brain, while the decline in male brain is steeper and linear from 20 to 90 years of age (Table 2 and Fig. 2A, B). The concentration of cerebroside and cholesterol was also higher in male than female brain from 20 to 39 years of age, indicating that there is a larger proportion of myelin in male than in female brain. We are not aware of any previous study in which the content of biochemical constituents of brain has been followed with age. Bürger [9] and Rouser and Yamamoto [10] also studied the changes in brain constituents with age, but they determined only their concentrations. We have also expressed our results as concentration of brain constituents to make comparison possible with the two previous studies. Rouser and Yamamoto [11] applied excellent methods developed by Rouser over many years, but they did not give exact figures for the concentrations. They only gave the equation to be used for the determination of the various lipids at a certain age. Horrocks et al. [21] have used their equations for calculations of the lipid concentrations of human brain at 40 years of age. Their values for the concentrations of phospholipids, cholesterol, cerebroside and sulfatide are very similar to ours (Table 3). This is a further support for our assumption that our data for the biochemical constituents of human brain are valid.

The ganglioside concentration did not show the same rapid decline as the other lipids. In female brain, it was constant between 20 and 70 years of age. This result corroborates our previous findings in frontal and temporal cortices, where we found the same ganglioside concentration at 70 as at 20 years of age [1]. In male brain, the ganglioside concentration decreased, but much less than that of other lipids. We have previously isolated myelin and crude synaptosomal fractions of human brain from young

and old subjects [18]. The compositions and concentrations of the various lipids including gangliosides were the same at all ages. These earlier findings and our present biochemical findings indicate that the neuronal membranes are far more resistant than myelin in female and male brain during ageing.

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