# A survey of human liver reserves of retinol in London

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1. The retinol content of human liver tissue, obtained at autopsy from mortuaries in the London area, was determined in a group of 364 subjects.

2. Tissue samples from the central portion of the right lobe of the liver were saponified, extracted with light petroleum (b.p. 40–60°) and retinol assayed colorimetrically using the trifluoroacetic acid procedure.

3. The frequency distribution of retinol reserves was skewed to the right (positive skewness). The mean retinol content for the entire group was 252 mg/kg, with a median of 198 mg/kg and a range of 0–1201 mg/kg. Of the subjects, 49% had reserves in the range 100–300 mg/kg (regarded as the 'normal range' for liver retinol) while 5% had reserves below 40 mg/kg (the arbitrary 'cut-off' point below which individuals are considered to be at risk). Only one subject had no detectable retinol reserves. On the other hand, 11% of the subjects had reserves exceeding 500 mg/kg.

4. No sex-related differences were apparent, the median retinol reserves of male and female subjects being 190 and 202 mg/kg respectively.

5. Median retinol reserves varied markedly with age. They were relatively low (121 mg/kg) in infancy, but were approximately three times greater in childhood, adolescence and young adulthood. Thereafter, reserves declined gradually with increasing age to a low of 113 mg/kg in subjects over the age of 90 years.

6. When subjects were classified according to primary cause of death, the median reserves were as follows (mg/kg): accidental deaths 270, cancer 207, cardiovascular diseases 204, intestinal diseases 178, nervous diseases 164, hepatic diseases 158, respiratory diseases 141, sudden infant death syndrome 127.

7. Retinol reserves in London are substantially higher than those reported from North America and are exceeded only by those reported from New Zealand and Ghana. It is therefore concluded, from the results of this survey and also from official estimates of the dietary intake of retinol and provitamin A carotenoids, that vitamin A status in London, and probably also in the rest of Britain, is quite satisfactory.

It is generally accepted that the most direct and accurate method of assessing vitamin A status is to measure the concentration of the vitamin in the liver, since it is in this organ that 90% or more of the body's reserves are located. However (and particularly from the viewpoint of field surveys) the tissue in question is usually inaccessible in living persons because, for ethical reasons, liver biopsies can be performed in only a limited number of clinical situations.

As an alternative, one can analyse samples of liver tissue from individuals soon after death. When such samples are obtained from individuals who have met sudden death from unnatural causes (such as road traffic accidents, gunshot wounds, etc.) and if a subsequent autopsy reveals that the individuals are apparently free from any gross pathological signs, then it seems reasonable to regard the liver retinol content of such individuals as being representative of healthy persons. One can then proceed to compare the retinol stores of such subjects with those from individuals whose deaths were caused by various diseases.

The first studies of human liver reserves of retinol were carried out in Britain in the 1930s and followed by similar studies in continental Europe and the United States (Moore, 1957). More recently, such studies have been carried out in New Zealand (Smith & Malthus, 1962); Ghana (Dagadu & Gillman, 1963); Canada (Hoppner *et al.* 1969); the United States (Underwood *et al.* 1970; Raica *et al.* 1972; Mitchell *et al.* 1973); Thailand (Suthutvoravoot & Olson, 1974) and Bangladesh (Abedin *et al.* 1976).

No surveys of retinol stores in Britain appear to have been carried out since the end of the Second World War. Great social and economic changes have occurred in this country

over the past 30 years or so, and it is only to be expected that such changes, of which the most important has undoubtedly been the establishment of the Welfare State, will be reflected in the nutritional status of the population. It thus seems an appropriate time to reassess vitamin A status in Britain. The results of such an assessment are presented in this paper.

## METHODS

### *Collection of specimens*

Tissue samples from a total of 364 subjects were analyzed. Liver specimens were obtained from two mortuaries, one in North London and the other in Central London. Information regarding age, sex and primary cause of death was obtained from these same sources, although for various reasons (mostly administrative) complete records were not available for approximately fifteen subjects. All samples were obtained between February 1977 and September 1978.

After arrival at the mortuary bodies were kept at 4° until autopsy. All samples of tissue were obtained within 12–96 h after death. It has been shown that, during this time interval, liver retinol levels are not affected (Suthutvoravoot & Olson, 1974).

Samples of liver tissue weighing 5–10 g were taken from the central portion of the right lobe of the liver. It has been demonstrated that tissue obtained from this particular site is representative of whole liver homogenates (Hoppner *et al.* 1968; Olson *et al.* 1979) and most workers in recent years have analyzed tissue from this site. The tissue specimens were placed in screw-capped, amber-glass containers and stored at −20° until analysed. It has been reported that in human liver tissue stored under such conditions, retinol is stable for at least 6 months (Parkinson & Gal, 1972). In the present study all samples were in fact analysed within 2 weeks of the death of the subject.

### *Estimation of retinol*

Portions (approximately 1 g) of liver tissue were saponified and extracted with light petroleum (b.p. 40–60°) by standard procedures (Roels & Mahadevan, 1967) and retinol was assayed by the trifluoroacetic acid procedure (Neeld & Pearson, 1963).

## RESULTS

### *Frequency distribution of retinol in human liver*

The frequency distribution (Table 1) was skewed to the right (positive skewness). The major statistical feature of such a distribution is that, as a consequence of the relatively few high values, the mean is appreciably greater than the median. For this reason it may be misleading to report mean values for retinol stores. Some workers have attempted to overcome this problem by making a logarithmic transformation of the data to produce a distribution which is less skewed (Mitchell *et al.* 1973). No attempt was made to do this in the present study since it was felt that such a statistical exercise would be unlikely to yield additional information of nutritional significance. Emphasis has instead been placed on the median values and also on the range of values obtained. If the mean has been used at all it is included only as an indicator of the extent of skewness of the data.

Table 2 summarizes the values obtained in this study with respect to retinol stores regardless of age, sex or cause of death. The major feature of the retinol stores was their extraordinary spread, with values ranging from 0 (non-detectable) to 1201 mg/kg. The person with no detectable reserves (incidentally, the only such individual in the entire survey) was a woman aged 86 years who died as a result of cancer, whilst the highest value was obtained from a woman aged 22 years who had committed suicide by taking an acute overdose of barbiturates.

Table 1. *Frequency distribution of liver retinol reserves in London (for a total of 364 subjects)*

| Retinol (mg/kg) | No. of subjects | Percentage of total |
|---|---|---|
| 0–9 | 4 | 1·1 |
| 10–39 | 15 | 4·1 |
| 40–99 | 53 | 14·6 |
| 100–199 | 114 | 31·3 |
| 200–299 | 66 | 18·1 |
| 300–399 | 48 | 13·2 |
| 400–499 | 24 | 6·6 |
| 500–599 | 17 | 4·7 |
| 600–699 | 7 | 1·9 |
| 700–799 | 5 | 1·4 |
| 800–899 | 5 | 1·4 |
| 900–999 | 3 | 0·8 |
| 1000–1099 | 1 | 0·3 |
| 1100–1199 | 1 | 0·3 |
| 1200–1299 | 1 | 0·3 |

Table 2. *Summary of values for retinol reserves in London (for a total of 364 subjects)*

| | |
|---|---|
| Mean (mg/kg) | 252 |
| Median (mg/kg) | 198 |
| Range (mg/kg) | 0–1201 |
| < 40 mg/kg (%) | 5·2 |
| 100–300 mg/kg (%) | 49·4 |
| > 500 mg/kg (%) | 11·0 |

Nearly half the subjects had reserves in the range 100–300 mg/kg, which has been suggested as the 'normal range' for liver retinol (Pearson, 1967).

Nineteen subjects (5·2% of the survey population) had reserves below 40 mg/kg, the arbitrary 'cut-off' point below which individuals are considered to be 'at-risk' (Hoppner *et al.* 1968). As mentioned previously, only one individual had no detectable retinol reserves while three others had reserves less than 10 mg/kg and fifteen individuals had reserves in the range 10–39 mg/kg.

At the other end of the range forty subjects (11·0% of the survey population) had reserves above 500 mg/kg, and three of these individuals had reserves in excess of 1000 mg/kg.

### *Retinol reserves in relation to sex*

There was no difference in the median retinol stores of males and females (Table 3) although the range of values for females was slightly greater. A somewhat higher proportion of females had 'low' reserves (less than 40 mg/kg) but on the other hand there were also proportionately more females with 'high' reserves (greater than 500 mg/kg).

### *Retinol reserves in relation to age*

The effect of age on median retinol stores (Table 4) revealed a pattern of relatively low levels in infancy (0–1 years) followed by a threefold rise in childhood (1–9 years) which persisted into adolescence (10–19 years) and young adulthood (20–29 years). From the fourth to the

Table 3. *Retinol reserves in London in relation to sex*

| | Males | Females |
|---|---|---|
| No. of subjects | 214 | 136 |
| Median (mg/kg) | 190 | 202 |
| Range (mg/kg) | 8–1042 | 0–1201 |
| Low reserves* (%) | 3·3 | 6·6 |
| High reserves† (%) | 9·3 | 13·2 |

* < 40 mg/kg. † > 500 mg/kg.

Table 4. *Retinol reserves in London in relation to age*

| Age group (years) | No. of subjects | Retinol (mg/kg) | | Subjects with low reserves* | | Subjects with high reserves† | |
|---|---|---|---|---|---|---|---|
| | | Median | Range | No. | % | No. | % |
| 0–1 | 13 | 121 | 8–320 | 1 | 7·7 | 0 | — |
| 1–9 | 10 | 358 | 178–575 | 0 | — | 1 | 10·0 |
| 10–19 | 11 | 348 | 101–615 | 0 | — | 1 | 9·1 |
| 20–29 | 12 | 331 | 57–1201 | 0 | — | 4 | 33·3 |
| 30–39 | 13 | 179 | 103–495 | 0 | — | 0 | — |
| 40–49 | 33 | 227 | 10–916 | 1 | 3·0 | 9 | 27·2 |
| 50–59 | 44 | 223 | 9–814 | 2 | 4·5 | 6 | 13·6 |
| 60–69 | 77 | 188 | 23–757 | 7 | 9·1 | 4 | 5·2 |
| 70–79 | 81 | 181 | 18–1132 | 3 | 3·7 | 6 | 7·4 |
| 80–89 | 43 | 175 | 0–1042 | 5 | 11·6 | 7 | 16·3 |
| > 90 | 12 | 113 | 48–640 | 0 | — | 1 | 8·3 |

* < 40 mg/kg. † > 500 mg/kg.

ninth decades of life, median reserves were approximately at the level of the over-all median (i.e. 198 mg/kg, see Table 2) although a definite downward trend could be detected from the fifth decade of life onwards. Finally, in very old people, the median stores were at the same level as in infancy. It is necessary to emphasize, however, that the preceding generalizations mask the fact that there was a great deal of overlap in the range of values for retinol stores among all age-groups.

With the exception of the age-groups 0–1 and 60–69 years, the number of subjects in each group who had high reserves exceeded the number with low reserves. Indeed, between the ages of 1 and 39 years, there was not a single subject with low retinol stores. The highest proportion of samples with reserves below 40 mg/kg came from subjects in the age-group 80–89 years, but even in this category there were more subjects with high reserves. The oldest person in the survey, a woman aged 102 years, had retinol stores of 205 mg/kg.

*Retinol reserves in relation to cause of death*

Median retinol stores (Table 5) were highest in individuals who had met sudden, accidental death (the 'control group' of this study). Subjects whose deaths were due to cardiovascular diseases (the great majority of them victims of heart attacks) or cancer (all types) had median reserves which were approximately 25% lower than those of accident victims. Subjects who died as a result of respiratory diseases (bronchitis and bronchopneumonia) had median stores which were approximately half the control values. The remaining subjects died as

Table 5. *Retinol reserves in London in relation to cause of death*

| Cause of death | No. of subjects | Retinol (mg/kg) | | Subjects with low reserves* | | Subjects with high reserves† | |
|---|---|---|---|---|---|---|---|
| | | Median | Range | No. | % | No. | % |
| Accident | 58 | 270 | 57–1201 | 0 | — | 8 | 13·8 |
| Cancer | 30 | 207 | 0–814 | 3 | 10·0 | 4 | 13·3 |
| Cardiovascular diseases | 169 | 204 | 10–1132 | 4 | 2·4 | 18 | 10·7 |
| Intestinal diseases | 11 | 178 | 26–650 | 1 | 9·1 | 2 | 18·2 |
| Nervous diseases | 5 | 164 | 110–640 | 0 | — | 1 | 20·0 |
| Hepatic diseases | 7 | 158 | 9–295 | 2 | 28·6 | 0 | — |
| Respiratory diseases | 61 | 141 | 6–1042 | 9 | 14·8 | 6 | 9·8 |
| Sudden infant death syndrome | 7 | 127 | 107–416 | 0 | — | 0 | — |

* < 40 mg/kg. † > 500 mg/kg.

a result of intestinal diseases (duodenal ulcers, peritonitis, etc.), nervous diseases (epilepsy), hepatic diseases (alcoholic cirrhosis of the liver) and the sudden infant death syndrome. In these latter categories the median retinol stores were of the order of 35–55% below control values. It is again necessary to emphasize that there was a large amount of overlap in the range of values for retinol stores in all the previously-mentioned categories. Only in subjects dying as a result of diseases of the respiratory and hepatic systems did the number of individuals with low retinol stores exceed those with high reserves.

## DISCUSSION

The highly-skewed frequency distribution of retinol stores (Table 1) is in accord with most reports in the literature. One of the problems associated with the interpretation of data on retinol stores is to decide what constitutes the 'normal range'. Pearson (1967) suggested that normal values for liver retinol lie in the range 100–300 mg/kg, but this seems inappropriate in view of the wide range of retinol stores compatible with health. Thus, the liver reserves of accident victims in this survey spanned a twentyfold range (57–1201 mg/kg) and a similarly large spread of the values has been found by other workers (Hoppner *et al.* 1969; Underwood *et al.* 1970; Suthutvoravoot & Olson, 1974).

Similar uncertainty exists with regard to the question of what ought to be the 'cut off' point for retinol stores below which individuals could be at risk of developing clinical symptoms of vitamin A deficiency. Canadian workers (Hoppner *et al.* 1968) first suggested that reserves below 40 mg/kg are indicative of a vitamin A status that is less than adequate, but the validity of this argument has been questioned by Suthutvoravoot & Olson (1974), who have calculated that a child with initial retinol stores of 40 mg/kg could consume a vitamin A-deficient diet for approximately 150 d (the so-called 'protection period') before clinical symptoms of deficiency appeared. These workers have put forward persuasive arguments in favour of their contention that it is more reasonable to adopt 'cut-off' points of 20 mg/kg for children and 10 mg/kg for adults, which would ensure in each instance a protection period of approximately 100 d.

On the other hand, there is evidence from clinical studies (McLaren, 1966) that retinol stores below 10 mg/kg are often associated with clinical signs of vitamin A deficiency. Furthermore, animal experiments have indicated (Rietz *et al.* 1974) that unless retinol stores are already at a pre-existing level of approximately 18 mg/kg, a new dose of retinol is extensively catabolized and poorly stored. Assuming that a similar mechanism is in operation in humans, it is not difficult to visualize a situation where a child with reserves

of 20 mg/kg or less is consuming a diet that is barely adequate in retinol. If, under such conditions, newly-absorbed retinol is extensively degraded, liver reserves might not be accumulated to 'safe' levels. Should the child succumb to infections or other stresses at this stage, clinical symptoms of deficiency could soon appear.

In the absence of further information, therefore, it seems preferable to err on the side of caution and to continue to regard retinol stores below 40 mg/kg as being indicative of 'low' or 'marginal' vitamin A status. By this criterion, the incidence of low retinol stores in London (5·2% of the survey population) is among the lowest reported from any part of the world. By contrast, the incidence of low reserves in North America is of the order of 12–40%, depending on the location. In Canada, 10% of the survey population had no detectable retinol stores at all (Hoppner *et al.* 1969). In Bangladesh (a country where vitamin A deficiency is a serious public health problem) 78% of the survey population had reserves below 40 mg/kg (Abedin *et al.* 1976).

The similarity in median retinol stores between males and females (Table 3) is in accord with several reports (Hoppner *et al.* 1969; Raica *et al.* 1972; Suthutvoravoot & Olson, 1974). Values for Washington, DC (Mitchell *et al.* 1973) indicate sex-related differences only in certain age-groups.

The pattern of variation in median retinol stores with age (Table 4) differs in at least two ways from that found in other countries. First, whereas median reserves in London appear to decline in the later years of life, the opposite trend seems to be evident in North America (Hoppner *et al.* 1969; Raica *et al.* 1972; Mitchell *et al.* 1973) and also in Asia (Suthutvoravoot & Olson, 1974; Abedin *et al.* 1976). The reasons for this discrepancy cannot be adequately explained at the present time. However, the important thing to note is that vitamin A status among the elderly in London appears to be satisfactory. Among subjects over the age of 60 years, 7% had low reserves whereas 8% had high reserves.

Secondly, while median reserves in London follow the trend that is apparent in other Western countries and reach a peak in childhood, it is noteworthy that they remain at this high level throughout adolescence and young adulthood. In North America, on the other hand, median stores in adolescence show an appreciable decline from the peak values reached in childhood. This phenomenon is particularly evident in Canada (Hoppner *et al.* 1969). In the two reports from Asia, retinol stores appear to increase steadily throughout the life-span of individuals, and there is no pronounced accretion of reserves in childhood.

Most workers have ascribed the preceding patterns of retinol storage in childhood to the practice, said to be widespread in Western countries, of giving multivitamin supplements to children during the primary school years. In Asian countries, on the other hand, it is the custom for the adults in the family, who are also the breadwinners, to receive the major share of the most nutritious foods in the household. Children may not be given the same priority, so that their vitamin A status only improves as they get older and can fend for themselves.

The median retinol reserves of individuals who met sudden, accidental death was 270 mg/kg (Table 5), a value exceeded only by those reported from New Zealand (Smith & Malthus, 1962) and Ghana (Dagadu & Gillman, 1963). In his pioneering studies of liver reserves in Britain, Moore (1937) reported that the median reserve in his sample of accident victims was 66 mg/kg, so there seems little reason to doubt that there has been a substantial improvement in vitamin A status in Britain over the past four decades. This conclusion is reinforced by a recent report from Edinburgh (McLaren *et al.* 1979) in which the median retinol reserve of fifty-nine victims of accidental death was 231 mg/kg, a value which is in good agreement with that obtained in the present study.

Among accident victims in London, there was not a single individual with reserves below 40 mg/kg. By contrast, 35% of accident victims in New York City (Underwood *et al.* 1970) and 19% in Canada (Hoppner *et al.* 1969) were in this category.

Interpretation of the retinol stores of individuals who died as a result of various diseases (Table 5) must take into account the fact that the median ages of these subjects were in the range 60–69 years, whereas the median age of accident victims was approximately 30 years. As already discussed, retinol stores in London tend to decline with age. Thus, the median reserve of subjects in the age-group 60–69 years was 188 mg/kg, which is not very different from the median reserves of subjects dying as a result of cancer (207 mg/kg) or cardiovascular diseases (204 mg/kg). On the basis of these results, therefore, it would seem that vitamin A status is essentially unaffected in cancer and cardiovascular diseases.

Post-mortem studies of retinol stores in cancer patients have produced conflicting results. In comparison with the median reserves of accident victims, reserves in cancer are lower by approximately 23% in Canada (Hoppner *et al.* 1969) and by 33% in New Zealand (Smith & Malthus, 1962). In the Canadian study, 26% of cancer victims had reserves below 40 mg/kg while 11% had no detectable stores at all. However, Mitchell *et al.* (1973) in Washington, DC could find no differences in median retinol stores of accident and cancer victims, and whereas 17% of the accident victims had low reserves (defined by these workers as those with reserves of less than 50 mg/kg) only 11% of the cancer patients were in this category. In a five-State survey in the United States, Raica *et al.* (1972) reported that retinol stores of accident victims were in fact lower than those whose deaths were due to disease-related causes, although no specific data for cancer were given.

The median retinol reserve of subjects dying as a result of respiratory diseases was approximately half that of accident victims (Table 5) and even when the effect of age is taken into account it could well be that such diseases are a drain on retinol stores. Almost 15% of these subjects had reserves below 40 mg/kg and two individuals had reserves of only 6 and 8 mg/kg. On the other hand, approximately 10% of such subjects had reserves greater than 500 mg/kg and the third highest value obtained in this study (1042 mg/kg) came from a subject with respiratory disease. Clearly, it is by no means inevitable that disease will have a deleterious effect on the accumulation of hepatic reserves, as long as dietary intake of the vitamin is adequate.

One of the disadvantages of post-mortem studies of liver reserves is that no information is available for the subjects' dietary intake of retinol and provitamin A carotenoids, which is the major determinant of the size of the reserves. In Britain, the annual reports of the National Food Survey are indirect sources of such information. The relevant report for the present study (Ministry of Agriculture, Fisheries and Food, 1978) gives a value of 1470 $\mu$g retinol equivalents (RE) as the mean national daily intake per person. This value, which is almost twice the recommended daily intake of 750 $\mu$g RE (Department of Health and Social Security, 1969), includes the contributions of both preformed retinol and provitamin A carotenoids but does not take into account intake of the vitamin either outside the home or in the form of pharmaceutical preparations.

An important feature of retinol consumption in Britain is the fact that regional variations do not appear to be significant, with lowest mean daily intakes in the West Midlands (1360 $\mu$g RE) and highest intakes in the Southwest (1560 $\mu$g RE). The mean intake for the Greater London area is 1460 $\mu$g RE. It will be recalled that the median retinol stores of accident victims in London and Edinburgh are very similar (270 and 231 mg/kg respectively) and the combination of dietary and biochemical evidence would seem to indicate that vitamin A status throughout Britain is not only rather uniform, but also quite satisfactory.

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