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Exposure to toluene

Concentration in subcutaneous adipose tissue

by Anders Carlsson, MD, Elisabeth Ljungquist¹

CARLSSON A, LJUNGQUIST E. Exposure to toluene: Concentration in subcutaneous adipose tissue. *Scand j work environ health* 8 (1982) 56-62. Twelve male subjects were exposed to a toluene concentration of about 300 mg/m³ in the inspiratory air during rest and/or physical exercise on a bicycle ergometer. Each subject was exposed during four 30-min periods. Before exposure the subjects' body fat was calculated by means of underwater weighing and skeletal measurements. Needle biopsies of subcutaneous adipose tissue were taken up to 12 d after the exposure was concluded. The concentration of toluene in the adipose tissue was determined by gas chromatography after evaporation into nitrogen at a high temperature. After exposure at rest for 2 h, the mean concentration of toluene in adipose tissue was 0.7 mg (7.7 µmol)/kg. The corresponding value after 2 h of work at 50 W was 9.9 mg (109 µmol)/kg. There was a declining concentration of toluene in adipose tissue with an increasing degree of obesity. The half-time for toluene in adipose tissue ranged between 0.5 and 2.7 d. It increased with increasing amounts of body fat.

Key terms: arterial blood, body fat, exercise, human, needle biopsy, rest, uptake.

Once a solvent has entered the blood stream, it becomes distributed to various tissues and organs in the body. The amount of a solvent distributed to the tissue of each organ is determined by the blood flow through the organ and the capacity of the organ to hold and metabolize the solvent.

Lipid solubility may be a major determinant of how much solvent is absorbed across cell membranes. Most solvents, for instance, enter the brain very rapidly because of their lipid solubility. However, lipid solubility may have another profound effect in terms of the storage of a solvent in adipose tissue depots. Since body fat normally constitutes about 10 % of the body weight of men (11), and more in obese humans, fat depots may hold a

significant proportion of the total solvent content of the body. Previous studies (8, 9, 10) have reported an accumulation of xylene and styrene in the adipose tissue of 5 and 8 % of the uptake, respectively, after short-term exposure.

In the present investigation, the intention was to study the concentration of toluene in subcutaneous adipose tissue after standardized exposure during exercise and rest. Another objective was to analyze the importance of different factors involved in the distribution of solvents to adipose tissue.

Subjects and experimental design

The subjects were 12 healthy men, 22 to 43 years of age. Before exposure the amount of body fat was calculated by means of underwater weighing and skeletal measurements (6, 7). The original method was modified in the present study, as the pulmonary residual volume was determined

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by means of the usual helium dilution method, instead of in water in connection with hydrostatic weighing. Agostoni et al (1) have reported a mean decrease of 16.7% in the residual volume when the determination was made with the subject submerged in water. The amount of body fat was compensated for by the present modification of the residual volume determination according to the report of Agostoni et al (1).

The subjects were exposed to a toluene concentration of 306 ± 13 (SD) mg/m³ (about 80 ppm) in the inspiratory air during four consecutive 30-min periods. They were exposed according to the following three alternatives: series I: four periods at rest (4 subjects); series II: four periods at 50 W (3 subjects); series III: rest, 50 W, 100 W, and 150 W (6 subjects). One subject participated in both series I and III with a three-month interval.

Arterial blood samples were taken every fifth minute during exposure and more frequently during the start of exposure and the change of work loads.

After the exposure was concluded, a 4-h monitored period of elimination followed. The elimination phase was standardized with regard to rest and physical exercise. Physical exercise consisted of treadmill walking (5 min every half hour). The treadmill velocity and inclination were selected so that the load on the respiratory and circulatory organs was equivalent to a work load of about 50 W on the bicycle ergometer. Lunch was taken 1 h after the conclusion of exposure.

Adipose tissue biopsies were taken prior to exposure in order to reveal substances interfering with the solvent analyses. Biopsies were also performed 0.5, 1, 2, 4, and 20 h after exposure. Further biopsies were performed up to 12 d after the exposure was concluded. The adipose tissue biopsies were taken from the upper lateral gluteal quadrant after intracutaneous anesthesia with a 1% lidocaine solution (Xylocain, Astra). The needle was lance-shaped, and the dimensions were 2.0 × 80 mm (KN 1480 W, Mediplast AB). The needle was connected via a Luer-Lok to a 50-ml syringe with a glass barrel and plunger (B-D Yale, Becton, Dickinson & Co). For details of the method, see the report of Engström & Bjurström (8). From each subject five

biopsies were taken from each gluteal region, including double biopsies. The puncture channels were kept as far apart as possible so that samples from tissue with circulation disturbed by a previous puncture would be avoided.

Methods

The concentrations of toluene in subcutaneous adipose tissue were determined by gas chromatography after evaporation of the solvent at 150°C into nitrogen, which was continuously exchanged. The evaporation lasted 60 min, and the gas was collected in 30-ml glass syringes. Gas samples were analyzed by a gas chromatograph (Carlo-Erba model 2350) equipped with a stainless steel column (LB 550 X, length 50 m, inner diameter 0.5 mm). The column temperature was 72°C, and the flow rate of the carrier gas nitrogen was 4 ml/min. The toluene concentrations were calculated on the basis of standard air samples with known toluene concentrations. The specimens were assayed in immediate conjunction with the sampling. The error of the method for a single determination was calculated to $\pm 12\%$ of the mean value on the basis of 15 double determinations with different puncture channels and concentrations ranging from 0.3 (3.3 μ mol) to 16.8 mg (185 μ mol)/kg.

During a review of the analysis results from all adipose tissue specimens taken at the laboratory, it was observed that reliable results were not obtained under the following circumstances: (i) blood on the specimen, (ii) specimen weight < 10 mg, (iii) specimen with more than a 15% content of water. From a total of 130 adipose tissue specimens (13 subjects × 10 specimens), 11 specimens were rejected because of one of these three reasons. The mean weight of the remaining 119 specimens was 45.5 mg (range 10.2–135.9 mg).

In order to check the reliability of the gas chromatographic method, two supplementary studies were performed.

In the first study a male rat (Sprague-Dawley, 200 g) was exposed to about 80 ppm of radioactively labeled toluene (1.1 MBq/mmol) in the inspiratory air for 2 h. The rat was killed immediately after the conclusion of exposure. Samples were taken from the subcutaneous adipose tis-

sue, and eight of them were analyzed by liquid scintillation counting (5), and eight by the gas chromatographic method. The mean weight of the 16 samples was 49 mg, ranging from 17 to 87 mg. The mean toluene concentration of the eight samples analyzed by gas chromatography was 11.6 mg (127 μmol)/kg (SD = 1.6). After evaporation, the samples were analyzed by liquid scintillation. There was no radioactivity left in the samples; therefore all the toluene had evaporated from the samples with the gas chromatographic method.

The mean concentration of the eight samples analyzed by liquid scintillation was 16.0 mg (176 μmol)/kg (SD = 2.5). The mean value from the gas chromatographic analyses was 72.3 % of the corresponding value from liquid scintillation counting, the difference being significant (Mann-Whitney test, $p < 0.01$).

In the second study 16 samples from subcutaneous adipose tissue of a rat were placed in separate 5-ml glass bottles. Each bottle was sealed with a teflon stopper and 0.350 μg of radioactive toluene (1.1 MBq/mmol) was added. Then the bottles were left for equilibration during 24 h.

After 24 h, the total amount of toluene

in the headspace and in the adipose tissue specimen was analyzed for each bottle. Eight of the samples were analyzed by liquid scintillation, and eight by gas chromatography. The mean weight of the 16 samples was 32 mg (range 11–61 mg). The total mean amount of toluene from the headspace and the sample, analyzed by gas chromatography, was 0.339 μg (SD = 0.018). The corresponding value according to liquid scintillation was 0.358 μg (SD = 0.012). There was no significant difference between the values from the gas chromatographic method and the liquid scintillation counting, or between the amount of toluene added to the bottles and the total amount in the headspace and specimen, after 24 h of equilibration (Mann-Whitney test, $p > 0.05$). Thus, there was no measurable loss of toluene during the period of analysis with the gas chromatographic method.

The difference between the gas chromatographic method and liquid scintillation counting *in vivo* may be explained by the presence of polar toluene metabolites in the water content of adipose tissue (3).

For the uptake measurements and the determination of concentrations in arterial

Table 1. Body height, body weight, and estimated amount of body fat (in kilograms and as percentage of body weight).

Series	Number of subjects	Body height (cm)		Body weight (kg)		Estimated amount of body fat (kg)		Estimated fat (% of body weight)	
		Mean	Range	Mean	Range	Mean	Range	Mean	Range
I	4	181	174–188	89.7	70.1–132.0	19.6	4.7–58.2	17.5	6.8–43.4
II	3	179	175–185	71.1	63.0–79.0	10.2	8.1–12.0	14.1	12.5–16.8
III	6	176	173–180	76.7	69.0–99.8	9.6	1.3–25.5	11.8	1.7–26.1

Table 2. Concentrations of toluene in arterial blood at the end of exposure and in adipose tissue 30 min after the conclusion of exposure. The quotients between these concentrations are also given. Exposure was performed with a toluene concentration of about 300 mg/m³ in inspiratory air during four consecutive 30-min periods. Series I: four periods at rest (4 subjects); series II: four periods at 50 W (3 subjects); series III: rest, 50 W, 100 W, and 150 W (6 subjects). Mean values and standard errors of the means are given. (1 mg of toluene = 11 μmol)

Series	Arterial blood (mg/kg)	Adipose tissue (mg/kg)	Quotient adipose tissue/arterial blood
I	0.6 $\pm$ 0.0	0.7 $\pm$ 0.2	1.2 $\pm$ 0.1
II	2.1 $\pm$ 0.1	9.9 $\pm$ 2.3	4.7 $\pm$ 1.3
III	3.3 $\pm$ 0.2	6.9 $\pm$ 2.0	2.1 $\pm$ 0.6

blood, the reader is referred to the report by Carlsson (4).

Results

In table 1 the anthropometric data of the subjects are listed. The mean values for both body weight and estimated amount of body fat (in kilograms and as percentage of body weight) were the highest in series I because of an extremely overweight subject, weighing 132 kg. In series III there was also an overweight subject, weighing about 100 kg. Except for these two subjects, there were no significant differences (Mann-Whitney test, $p > 0.05$) between the series with respect to body height, weight, and estimated amount of body fat.

The concentration of toluene in the subcutaneous adipose tissue ranged from 0.1 mg (1.1 μmol) to 18.0 mg (198 μmol)/kg. The mean concentration of toluene in adipose tissue was 6.7 mg (73.8 μmol)/kg 4 h after the exposure at work with increasing work loads (series III) was concluded (fig 1). It had declined to 1.6 mg (17.2 μmol)/kg 4 d later. The concentrations after exposure at 50 W (series II) were consistently higher compared to the corresponding values after exposure at work with increasing work loads. After concluded exposure at rest for 2 h (series I), the mean concentration of toluene in adipose tissue was about 10 % of the corresponding value after exposure at work with increasing work loads. The noted dif-

ferences between the three series were followed up to 20 h after the end of exposure.

The quotients between the concentrations of toluene in adipose tissue 30 min after the end of exposure and the concentrations in arterial blood at the end of exposure were calculated (table 2). The concentrations of toluene in adipose tissue varied to a small extent during the first 4 h after the conclusion of exposure (fig 1). The highest quotient, 4.7, was determined, after 2 h of exposure at 50 W, while the lowest, 1.2, was found after 2 h of exposure at rest. In all the series there was a tendency for the quotients to drop with increasing amounts of body fat in the subjects.

For the subjects in series III, a linear regression analysis disclosed a declining concentration of toluene in adipose tissue with an increasing degree of obesity (fig 2).

If it is assumed that the concentrations of toluene in individual specimens are representative of the mean concentration in adipose tissue, the total amount of toluene in the adipose tissue can be calculated. The total amount of adipose tissue can be estimated from the body fat weight on the assumption that the average fat content of adipose tissue is 80 % (11). Table 3 lists, for all the subjects in series I, II, and III, the estimated total amount of toluene in the adipose tissue, as the percentage of total uptake of toluene, at different times after the exposure was concluded. After exposure during both rest and exercise, the ratio between the estimated amount of

Fig 1. Concentrations of toluene in the subcutaneous adipose tissue up to 20 h after the conclusion of exposure. Exposure was performed with a toluene concentration of about 300 mg/m³ in inspiratory air for four consecutive 30-min periods during rest and exercise. Series I: four periods at rest (4 subjects); series II: four periods at 50 W (3 subjects); series III: rest, 50 W, 100 W, and 150 W (6 subjects). Mean values and standard errors of the means are stated. (1 mg of toluene = 11 μmol)

toluene in adipose tissue and the total amount taken up seemed to increase with an increased degree of obesity. For the leanest subject, the amount of toluene in the subcutaneous adipose tissue, 1 h after the conclusion of exposure, was about 5 % of the total uptake. The corresponding value for the most obese subject was 20 %. Three days after the exposure was concluded, the value for the leanest subject had declined to 0.0, whereas the value for

the obese one was approximately 12 %. The values of subject number 5 differed from the others. He had higher values than expected.

The concentrations of toluene in subcutaneous adipose tissue were measured up to 12 d after the exposure was concluded. The relationship between the half-time for toluene in subcutaneous adipose tissue and the amount of body fat, as the percentage of body weight, was linear, and

Table 3. Estimated body fat as percentage of body weight, total uptake of toluene, and estimated amounts of toluene in adipose tissue (as percentage of total uptake of toluene) up to 8 d after the conclusion of exposure. Exposure was performed with a toluene concentration of about 300 mg/m³ in inspiratory air during four consecutive 30-min periods. Series I: four periods at rest (4 subjects); series II: four periods at 50 W (3 subjects); series III: rest, 50 W, 100 W, and 150 W (6 subjects). (1 mg of toluene = 11 μ mol)

Subject	Estimated fat (% of body weight)	Uptake (mg)	Estimated amount of toluene in adipose tissue at different times after the conclusion of exposure (as percentage of total uptake of toluene)				
			1 h	4 h	20 h	3 d	8 d
<i>Series I</i>							
1	6.8	137	2.3	3.2	3.5	0.5	0
2	8.2	175	1.5	2.6	2.6	2.1	0.5
3	11.7	169	5.0	7.4	5.6	3.6	0.7
4	43.4	225	24.3	17.3	17.3	24.3	2.6
<i>Series II</i>							
5	12.5	396	44.9	49.8	29.3	8.7	1.0
6	13.0	444	21.0	18.3	18.4	8.5	0.9
7	16.8	438	21.6	13.3	11.3	5.5	0.8
<i>Series III</i>							
8	1.7	473	4.6	3.9	3.3	0	0
9	6.8	427	5.0	5.5	5.0	1.5	0
10	8.6	413	12.2	11.8	9.7	4.5	0.2
11	10.2	378	22.3	22.6	17.7	8.2	0
12	17.1	425	26.3	19.8	17.9	8.0	1.1
13	26.1	579	20.0	21.8	21.8	12.4	4.7

Fig 2. The concentration of toluene in the subcutaneous adipose tissue 30 min after the conclusion of exposure in relation to the amount of body fat (as percentage of body weight) for the subjects in series III. Exposure was performed with a toluene concentration of about 300 mg/m³ in inspiratory air for four consecutive 30-min periods during rest and exercise. Series III: rest, 50 W, 100 W, and 150 W (6 subjects). Regression line: $y = -0.07X + 2.38$; $r = -0.94$; (1 mg of toluene = 11 μ mol)

they closely correlated to each other (fig 3). The half-time increased with increasing amounts of body fat and ranged between 0.5 and 2.7 d.

Discussion

The concentrations of toluene in the subcutaneous adipose tissue were about 10 to 14 times higher after 2 h of exposure during exercise (series II and III) than at rest (series I) (fig 1). One reason for the great difference is that the arterial concentrations of toluene at the end of exposure during exercise were about three to five times higher than at rest. Another reason is the greater blood perfusion in subcutaneous adipose tissue during exercise than during rest (2).

Linear regression analysis disclosed a decreased concentration of toluene in adipose tissue with an increased degree of obesity. Nielsen & Larsen (12) reported that the blood perfusion in subcutaneous adipose tissue declines with increasing amounts of body fat. This phenomenon may explain the fact that toluene concentrations in adipose tissue are lower in subjects with large amounts of body fat.

When the total amounts of toluene in the adipose tissue were calculated, it was assumed that the concentrations in the gluteal region were representative of the concentrations in the total amount of adipose tissue. Naturally, this assumption means a theoretical construction. The distribution

of a solvent in human adipose tissue is probably not uniform. Bülow & Madsen (2) reported a ratio of 1.7 between the blood perfusion in perirenal and subcutaneous adipose tissue in man at rest, increasing to 3.2 at about 100 W.

The quotients between the concentrations of toluene in subcutaneous adipose tissue and arterial blood ranged from 1.2 after exposure at rest to 4.7 after exposure at 50 W (table 2). One reason for the higher quotient during exercise may be an increased blood perfusion (2). The quotients from the present study were much lower than the one calculated from *in vitro* experiments with oil/blood. Sato & Nakajima (13) reported an oil/blood partition coefficient of 94 for toluene. The main reason for the different values is that the present study was undertaken *in vivo*. It means, among other things, that subcutaneous fat is not in equilibrium with arterial blood after only 2 h of exposure. Another contributing factor is that subcutaneous fat cannot be equated with oil (11).

There was a linear correlation between elimination half-times of toluene in subcutaneous adipose tissue and the amount of body fat, as percentage of body weight.

In the present study, some factors important to a high concentration of toluene in subcutaneous adipose tissue have been illustrated: (i) exposure during physical exercise causes both an increased blood perfusion in the subcutaneous adipose tissue and an increased concentration of toluene in the arterial blood in comparison to

Fig 3. Half-time for toluene in subcutaneous adipose tissue after conclusion of exposure in relation to the amount of body fat. Exposure was performed with a toluene concentration of about 300 mg/m³ in inspiratory air for four consecutive 30-min periods. Series II: four periods at 50 W (3 subjects); series III: rest, 50 W, 100 W, and 150 W (6 subjects). Regression line: $y = 0.08 X + 0.41$; $r = 0.95$ (1 mg of toluene = 11 μ mol)

toluene concentrations under resting conditions and (ii) blood perfusion increases with declining amounts of body fat.

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