Nitrous oxide (N₂O) has been used for anaesthesia during neurosurgical procedures for half a century, as it was previously thought to be inert. However, in the 1970s, it was shown that N₂O had potentially striking effects on intracranial pressure (ICP). The literature regarding the effects of N₂O on the brain is still equivocal, probably due to species differences, but also because of interactions with other drugs or interventions. In humans, evidence supporting the conclusion that N₂O is a cerebral vasodilator in the absence of other interventions has been obtained from both two- and three-dimensional cerebral blood flow (CBF) studies.

Three-dimensional CBF measurements during N₂O 50% inhalation show that CBF increased in all regions, even though not evenly distributed in the brain. The reason for this increase in flow is still unknown, but it may theoretically be due to an increased cerebral metabolism or an influence of N₂O on the cerebral vessels, for instance through release of vasoactive mediators.

In order to explore the former hypothesis, the aim of the present study was designed to evaluate the effect of N₂O 50% on the cerebral metabolic rate for glucose (CMRglu) and its distribution using a three-dimensional positron emission tomography (PET) technique.

Methods
Ten male volunteers, age 25–40 yr (mean 31 yr), participated in the study. The ethics committee for human...
were done by starting with O2 30%. The data from the succeeding PET scans, all the rest of the investigations (four) and N2O 50%. Oxygen-enriched air was compressed air using a soft-tissue attenuation coefficient. The attenuation was calculated from contours cross slices. Corrections for random and scattered events were made. The attenuation calculation was based on the scanner’s specifications.

PET examinations were performed with a PC384-7B scanner (Scanditronix, Uppsala, Sweden). The four ring detector system generated four main slices and three cross slices, with an approximate slice thickness of 13 mm. The mean sensitivity of the scanner was 675 cps (kBq ml⁻¹)⁻¹ for the main slices and 945 cps (kBq ml⁻¹)⁻¹ for the cross slices. Corrections for random and scattered events were made. The attenuation was calculated from contours using a soft-tissue attenuation coefficient.

The 2-¹⁸FDG was synthesized using ¹⁸F from the Lund electrostatic tandem accelerator with a proton beam of 6.0 MeV and produced by the ¹⁸O(p,n)¹⁸F reaction,⁹ and by fluorination of the precursor 1,3,4,6-tetra-O-acetyl-2-O-trifluoro-methane-b-d-mannopyranose according to a method described by Toorongian and colleagues.¹⁰ The radiochemical purity of FDG was better than 95%.

PET images were acquired within 60 min after administration of 50–150 MBq FDG, with the highest dose at the second measurement, by a 2 min i.v. injection into a peripheral vein in the arm. Venous blood samples were drawn from the other arm at appropriate intervals for quantification (every 20 s for 4 min, every 60 s for 10 min, and every 5 min up to a total of 35 min). When the blood samples had been centrifuged, radioactivity in the plasma (Cₚ) was measured using a well counter. Blood glucose (Cₕ) values were obtained, from the same site, immediately before, 10 and 35 min after injection of FDG. Two FDG studies were made on each subject with a time interval of approximately 2 h. The three-dimensional metabolic rate of glucose in the brain was recorded, parallel to the orbito-meatal (OM) line, with the centre of the lowest slice located 1 cm below the OM line. The head position was controlled using light beams on the external auditory meatus and the nasion as landmarks. Each study consisted of two separate scans (2×10 min), in between which the couch was moved half a plane separation (6.75 mm) in order to get a higher axial sampling rate. The CMRglu were calculated using the test–retest method developed by Brooks and colleagues,¹¹ which takes into account the residual activity from the first investigation in the calculation of the second.

A number of blood samples were taken during a 35 min period after each FDG-injection, and the activity in plasma was measured. These curves were extrapolated by fitting a monoexponential function to the tail of the curve (the last 25 min). The first extrapolated curve was adjusted to match a sample taken immediately before the second injection. An example of a plasma activity curve is shown in Figure 1.

Tomographic images were reconstructed by filtered back projection using a Hanning reconstruction filter with a cut-off frequency of 2.1 cm⁻¹. The image matrix size was 128×128 and the pixel size 2.2 mm.

The calculation of the second measurement demanded that the images from the two studies were perfectly registered. This was accomplished first by carefully positioning the subjects in the same way for the two studies using line-laser for positioning, and secondly by shifting and rotating the images interactively.

Standard sets of regions of interest (ROIs) corresponding to the brain lobes, cerebellum, pons, thalamus, and basal ganglia were analysed by a ROI analysis program (Amersham, UK). The program works with anatomical templates from a CT brain atlas.¹² The templates were applied to the brain slices and scaled to the actual head.

**Fig 1** Representative curve from one participant demonstrating the activity of FDG in plasma as a function of time.
size according to the external brain diameters. Three-dimensional cerebral ROIs were calculated from adjoining ROIs in different brain slices representing the same structure. With the aid of the ROIs, the mean CMRglu in different brain regions and the global mean CMRglu was calculated.

The quantities of CO₂, O₂, and N₂O in the inspiratory and expiratory gas mixtures were continuously measured along with the pulse rate and arterial haemoglobin oxygen saturation (Sao₂), with an Ohmeda 4700 OxiCap (BOC Health Care, Louisville, KY, USA). Non-invasive arterial pressure was recorded with 5 min intervals (Colin Press-Mate, Colin Electronics, Japan) during the inhalation of gas. At the end of the study, a blood sample was withdrawn to measure the haemoglobin concentration.

Global effects of N₂O on the CMRglu were analysed by two factor repeated measures analysis of variance (ANOVA) with correction for departure from sphericity for the interaction data.¹³ As the investigations were test–retest situations with correction for departure from sphericity for the interaction data,⁴ significant ANOVA interactions were further analysed with Student’s paired t-test.

P≤0.05 was considered statistically significant. All values are given as mean (SD). All statistical analyses were performed using the Stat View program version 5.0.1 for Windows (SAS Institute, Cary, NC, USA).

Results

Physiological values for inhalation of oxygen-enriched air and for inhalation of N₂O 50% is presented in Table 1. The mean haemoglobin value was 140 g litre⁻¹, range 120–154 g litre⁻¹.

All volunteers experienced psychogenic changes in cerebral function during N₂O inhalation with reduction in wakefulness, vision, hearing, and touch, whereas there was an increase in impulsiveness.

The volunteers had a global CMRglu of 27 (3) μmol 100 g⁻¹ min⁻¹ when breathing oxygen-enriched air. Inhalation of N₂O 50% did not change global CMRglu 30 (5) μmol 100 g⁻¹ min⁻¹ significantly, whereas it induced a significant change in the distribution (F=7.7, P<0.0001) (Fig. 2, Table 2). PCA analysis identified the factors explaining 89% of the variance in the ROIs. These were high negative loading in frontal, parietal, and temporal cortex vs positive loading in pons; high negative loading in cerebellum and positive in thalamus as well and basal ganglia; and finally high negative loading in the occipital region. Only high negative loading in cerebellum and positive in thalamus as well and basal ganglia reached the level of significance (P<0.0001).

Comparing the individual ROIs, N₂O significantly increased metabolism in thalamus by 22 (23%) (t=2.59, P=0.01) and basal ganglia by 14 (17)% (t=2.20, P<0.05) (Fig. 3).

In the different ROIs, inhalation of N₂O 50% changed the metabolism as shown in Figure 3. Representative PET and SPECT scans (CBF) under the same conditions with and without N₂O are presented in Figure 4.

There was a significant difference of the results whether N₂O or oxygen-enriched air was inhaled first (F=5.48, P<0.0001). In the three volunteers who started with N₂O

<table>
<thead>
<tr>
<th>Table 1</th>
<th>Physiological variables during the two experimental conditions. Values are presented as mean (sd).</th>
<th>O₂ 30%</th>
<th>N₂O 50%</th>
</tr>
</thead>
<tbody>
<tr>
<td>n</td>
<td>10</td>
<td>10</td>
<td></td>
</tr>
<tr>
<td>End-tidal CO₂ (kPa)</td>
<td>5.0 (0.3)</td>
<td>4.5 (0.4)*</td>
<td></td>
</tr>
<tr>
<td>SaO₂ (%)</td>
<td>100 (0.3)</td>
<td>100 (0.3)</td>
<td></td>
</tr>
<tr>
<td>MAP (mm Hg)</td>
<td>87 (11)</td>
<td>92 (12)</td>
<td></td>
</tr>
<tr>
<td>Heart rate (beats min⁻¹)</td>
<td>64 (8)</td>
<td>66 (14)</td>
<td></td>
</tr>
</tbody>
</table>

*P<0.05
During inhalation of oxygen-enriched air, the global CMRglu of the human brain was 27 μmol 100 g⁻¹ min⁻¹ that is in accordance with former similar studies. In the present study, inhalation of N₂O 50% in healthy young men resulted in only a minor and non-significant increase in global CMRglu. This is in fact not a surprising finding as no human study has demonstrated any increase in CMR due to N₂O inhalation; previous studies were limited by the fact that another anaesthetic drug was present during the investigation and which has been used to explain the lack of effect of N₂O on CMR. In the study by Kaisti and colleagues, where adjunct of N₂O counteracts the CMRO₂ and CBF reduction by propofol and sevoflurane, the amounts of propofol and sevoflurane given is lowered when N₂O was added, in order to keep the anaesthetic level constant. The effect could be due to a reduction in the basic anaesthesia or an increase in CMR due to the N₂O addition. In support of this assumption, N₂O generally increased the CMR in animals if administered alone, but this increase in metabolism could be reduced or abolished by augmenting the anaesthetic level through addition of other anaesthetics. The animal studies, together with the fact that N₂O changes the EEG pattern in humans, have been used to postulate that N₂O...
increases CMR also in man, which our study does not support.

Compared with inhalation of oxygen-enriched air, N2O 50% inhalation made the participants hyperventilate with a significant lowering of the 𝑒′ 𝐶O2 by 0.5 kPa (4 mm Hg), which is in accordance with other studies. It is generally accepted that changes in CO2 affect the CBF, but not CMR which remains unaffected of CO2 changes within arterial CO2 levels of 2–10 kPa (15–80 mm Hg). One possible reason for the reduced effect of N2O on the CMRglu, as suggested by Stapleton and colleagues, can be explained by an adaptation to lower anxiety during the second PET scan. However, most of the participants in the present study had also participated in a former study evaluating the effect of N2O on the CBF, which would be expected to relieve the anxiety effect of these participants. Furthermore, N2O 50% inhalation has profound psychogenic effects, making it difficult to extrapolate the findings of Stapleton and colleagues to our experimental condition. We also noted that participants who started with inhalation of N2O 50% tended to increase global CMRglu during the succeeding inhalation of oxygen-enriched air. As volunteers inhaling air twice did not change their metabolism, this observation is best explained by residual effects of N2O on the succeeding CMRglu, and does not favour the view that CMR should generally be decreased at the second measurement.

Inhalation of N2O 50% has been reported to increase global CBF by 22–40% in man. This effect is mainly believed to be due to the mental activation by N2O as changes in rCBF should normally be directly coupled to changes in rCMR. In this study, inhalation of N2O 50% induced a non-significant increase in the global CMRglu of 10% (Fig. 4). This contrasts to the earlier findings where CBF increased by 22–40%, indicating that there might be a direct or indirect effect on the cerebral arteries in addition to the cerebral metabolic effect. In support of this theory, addition of N2O to an isoflurane anaesthesia during normocapnia resulted in an increased CBF without any influence on the rCBF pattern created by isoflurane. This uncoupling between CBF and CMR has not been found to be due to a direct effect on the human cerebral vessels by N2O, opening up for alternative explanations to this discrepancy.

Inhalation of N2O altered the regional distribution of CMRglu, which increased in thalamus and basal ganglia. Utilizing the same technique to identify ROIs, N2O inhalation was found to augment rCBF in most brain regions, although thalamus and basal ganglia were among the regions with the greatest change. Even though we do not find a complete match between flow and metabolism, such conformity has been verified for inhalation of N2O 20%. The correlation between flow and metabolism may therefore explain the regional cerebral flow pattern found during N2O inhalation, which, however, is generally increased (uncoupled) due to the higher concentration of N2O inhaled. The flow pattern gave the impression that N2O inhalation increased flow through regions anatomically associated with the limbic system. In support of the theory of limbic system activation by N2O, we have previously reported that N2O stimulated thoughts and emotions, which normally are dealt with by the limbic system. In addition, former reports have shown an increased EEG activity in the limbic regions in cats and in humans during N2O inhalation.

In conclusion, inhalation of N2O resulted in a small and non-significant increase in global CMRglu. This effect on the global metabolism was mainly attributed to an increased metabolism in thalamus and basal ganglia. The metabolic effect of N2O may linger in the brain more than 1 h after clinical recovery from anaesthesia.

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N\textsubscript{2}O and cerebral metabolism