Effects of ATP infusion on glucose turnover and gluconeogenesis in patients with advanced non-small-cell lung cancer


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ABSTRACT

Cancer cachexia is associated with elevated lipolysis, proteolysis and gluconeogenesis. ATP infusion has been found to significantly inhibit loss of body weight, fat mass and fat-free mass in patients with advanced lung cancer. The present study was aimed at exploring the effects of ATP on whole-body glucose turnover, alanine turnover and gluconeogenesis from alanine. Twelve patients with advanced non-small-cell lung cancer (NSCLC) were studied 1 week before and during 22–24 h of continuous ATP infusion. After an overnight fast, turnover rates of glucose and alanine, and gluconeogenesis from alanine, were determined using primed constant infusions of [6,6-2H2]glucose and [3-13C]alanine. Thirteen NSCLC patients and eleven healthy subjects were studied as control groups without ATP infusion. During high-dose ATP infusion (75 µg min⁻¹·kg⁻¹), glucose turnover was 0.62 ± 0.07 mmol h⁻¹·kg⁻¹, compared with 0.44 ± 0.13 mmol h⁻¹·kg⁻¹ at baseline (P = 0.04). For gluconeogenesis a similar, but non-significant, trend was observed [baseline, 0.30 ± 0.16 mmol h⁻¹·kg⁻¹; during ATP, 0.37 ± 0.13 mmol h⁻¹·kg⁻¹ (P = 0.08)]. At lower ATP doses (37–50 µg min⁻¹·kg⁻¹) these effects were not detected. The relative increase in glucose turnover during ATP infusion compared with baseline showed a significant correlation with the ATP dose (r = 0.58, P = 0.02). No change in alanine turnover was observed at any ATP dose. The results of this study indicate an increase in glucose turnover during high-dose ATP infusion compared with baseline levels. During high-dose ATP infusion, glucose turnover was similar to that during low-dose ATP infusion and to that in control NSCLC patients. Between ATP infusions, however, glucose turnover in patients treated with high-dose ATP was significantly lower than that in the low-dose and control NSCLC patients (P = 0.04 and P = 0.03 respectively), and similar to that in healthy subjects. This would suggest that repeated high-dose ATP infusions may inhibit glucose turnover between infusion periods.

INTRODUCTION

Cachexia is a common phenomenon in lung cancer patients, and contributes significantly to morbidity and mortality [1–3]. Cancer cachexia is associated with metabolic alterations, including elevated lipolysis [4,5], protein breakdown [6–8] and increased glucose turnover [9]. In patients with advanced cancer, increased glucose

Key words: ATP, gluconeogenesis, glucose turnover, lung cancer, patients.

Abbreviation: NSCLC, non-small-cell lung cancer.

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production has been shown from lactate [10], glycerol [11] and alanine [12,13].

It has been argued that the liver plays an important role in the metabolic alterations contributing to the development of weight loss in cancer [14,15]. In liver [16–18] and skeletal muscle [16] of tumour-bearing rats, significantly lowered ATP levels have been demonstrated, which were associated with increased gluconeogenesis [18] and increased gluconeogenic enzyme activity [19]. In mice bearing CT26 colon tumours, daily intraperitoneal injections of ATP, AMP or adenosine for 10 consecutive days significantly inhibited host weight loss [20]. This inhibition was associated with increased hepatic ATP pools [20,21].

In a randomized clinical trial in patients with advanced non-small-cell lung cancer (NSCLC), we recently demonstrated beneficial effects of ATP infusions on body weight, muscle strength [22], skeletal muscle mass and body cell mass [23]. The present pilot study was aimed at exploring the effects of ATP infusion on whole-body glucose turnover, alanine turnover and gluconeogenesis from alanine. Based on the beneficial clinical outcomes, we hypothesized that ATP would have an inhibitory effect on these processes. Alanine was selected as a gluconeogenic substrate because this amino acid is the key protein-derived precursor of glucose utilized by the liver [24], and a major component of muscle protein degradation.

METHODS
Subjects and study design
Patients with histologically or cytologically proven NSCLC (stage IIIB or IV without curative options, and a Karnofsky index of 60% or more) were eligible for the study. Patients with cognitive dysfunction or liver, renal, respiratory or heart failure, and patients undergoing surgery, concurrent chemotherapy or radiotherapy involving all lesions, were excluded. In addition, 11 healthy subjects and 13 NSCLC patients without ATP infusion were included as control groups. The control NSCLC patients were matched for the ATP-infused NSCLC patients for age, sex and weight loss. The study was approved by the Medical Ethical Committee of the Erasmus University Medical Centre Rotterdam. All participants provided written informed consent.

In the clinical trial, 28 patients were randomly allocated to ATP treatment, to receive a maximum of 10 ATP courses of 30 h each: seven courses at 2-week intervals, followed by three courses at 4-week intervals. ATP infusions (6.1 mg of ATP-Na$_2$·3H$_2$O in 1 ml of 0.9% NaCl) were started at an initial concentration of 20 μg·min$^{-1}$·kg$^{-1}$ and were increased by increments of 10 μg·min$^{-1}$·kg$^{-1}$ every 30 min to a maximum dose of 75 μg·min$^{-1}$·kg$^{-1}$, or until the maximally tolerated dose had been reached. If any side effects occurred, the dose was reduced to the last given dose, or further until the side effects disappeared, usually within minutes of lowering the ATP dose. Thereafter, ATP was infused at a continuous rate. The most frequently occurring side effects were chest discomfort and the urge to take a deep breath [25].

In 12 out of the 28 ATP-infusion patients, glucose turnover and gluconeogenesis from alanine were studied 1 week before (baseline) and during an ATP course (22–24 h after starting the ATP infusion). Seven patients received low-dose infusions of 37–50 μg·min$^{-1}$·kg$^{-1}$ ATP, and five received high-dose infusions of 75 μg·min$^{-1}$·kg$^{-1}$ ATP.

The subjects were studied in the morning after an overnight fast. A cannula (0.8 mm × 25 mm) was placed in the left cubital vein for the infusion of stable isotope tracers. In the contralateral cubital vein, an identical cannula was positioned for blood sampling. To study gluconeogenesis, a solution was prepared containing d-[6,6-2H$_2$]glucose (98 atom%) and l-[3-13C]alanine (99 atom%) (Mass Trace, Woburn, MA, U.S.A.) in water, and this was sterilized by autoclaving in glass vials. A priming dose of 0.03 mmol/kg d-[6,6-2H$_2$]glucose was administered, followed by a continuous infusion of 0.01 mmol·h$^{-1}$·kg$^{-1}$ d-[6,6-2H$_2$]glucose for 90 min. Simultaneously, a priming dose of 0.08 mmol/kg l-[3-13C]alanine was given, followed by a continuous infusion of 0.04 mmol·h$^{-1}$·kg$^{-1}$ l-[3-13C]alanine over 90 min. Both tracer solutions were infused using calibrated syringe pumps (Perfusor® fm; Braun).

Venous blood samples were drawn immediately before the isotope infusions were started, and at 10 min intervals from 30 to 90 min during the tracer infusions, i.e. after steady-state conditions had been achieved.

Analytical methods
Blood samples were collected in tubes containing lithium heparin (Vacutainer®; Becton Dickinson, Meylan Cedex, France) and immediately stored on ice. After centrifugation (10 min, 1200 g, 4 °C), the plasma was collected and stored at −20 °C until analysed. An aliquot of the infusate was analysed to document the actual concentrations of the tracers in each study.

Blood glucose concentrations were determined enzymically with a glucose oxidase/peroxidase assay system (Boehringer Mannheim, Mannheim, Germany). Plasma alanine was measured enzymically as described by Williamson [26]. Isotopic enrichments were determined using the following procedures. Plasma was deproteinized by adding 0.3 M BaOH (Sigma Diagnostics) and 0.3 M ZnSO$_4$ (Merck, Darmstadt, Germany). After centrifugation (8 min, 15000 g, 4 °C) the supernatant was applied to an ion-exchange column (mixed
bed: AG50W-X8 and AG1-X8; 200–400 mesh; 0.2 g of each; Bio-Rad). Glucose and alanine were eluted from the column using water and 4 M ammonium hydroxide (Merck) respectively, and dried under nitrogen.

A glucose derivative (aldonitrile penta-acetate) was prepared as described by Varma et al. [27]. An alanine t-butyldimethylsilyl derivative was prepared as described by Chaves Das Neves and Vasconcelos [28].

Isotopic enrichments were measured by injecting 1 µl samples with a split ratio of 50:1 on to a fused silica capillary column of 25 m x 0.22 mm, coated with 0.11 µm HT5 (SGE, Victoria, Australia). The relative isotopic enrichments of $[^3\text{H}]_\text{glucose}$ and $[^1\text{H}]_\text{alanine}$ were determined using a Carlo Erba GC8000 gas chromatograph coupled to a Fisons MD800 mass spectrometer (GC-MS) (Interscience B.V., Breda, The Netherlands) in electronimpact ionization mode. In general, the coefficient of variation for enrichment was 0.2 mol% for both $[^6,6,^2\text{H}]_\text{glucose}$ and $[^3,\text{C}]_\text{alanine}$ measurement, and no concentration effect was observed at this mol% enrichment level. Ions were monitored selectively at $m/z$ 187 for natural glucose and $m/z$ 189 for the deuterated molecule. The isotopic enrichment of $[^3,\text{C}]_\text{alanine}$ was determined at $m/z$ 260 and 261 for alanine containing $^{12}\text{C}$ and $^{13}\text{C}$ respectively [29].

Total enrichment of $[^1\text{C}]_\text{glucose}$ was measured separately (aldonitrile penta-acetate derivation) using a gas chromatograph combustion isotope ratio mass spectrometer (Optima; Micromass UK, Middlewich, Cheshire, U.K.). The $[^1\text{C}]_\text{glucose}$ enrichment in atom% excess was monitored after combustion to $\text{CO}_2$ at mass 44 for $^{12}\text{C}$ and mass 45 for $^{13}\text{C}$.

**Calculations**

The whole-body rate of appearance (Ra) of glucose was calculated during steady state employing a one-compartment model, using the equation:

$$Ra = F \times [(IE_i/IE_{ee}) - 1]$$

where $F$ is the isotopic infusion rate (mmol·h$^{-1}$·kg$^{-1}$), $IE_i$ the isotopic enrichment of the infusate (mol% excess) and $IE_{ee}$ the isotopic enrichment of the extracellular fluid (mol% excess) [30]. The percentage glucose produced from alanine is given by:

Glucose from alanine (%) = $\frac{IE[^{13}\text{C}]_\text{glucose Plasma}}{IE[^{13}\text{C}]_\text{alanine Plasma} \times 0.33}$

The correction factor in eqn. (2) is applied in order to correct for the number of carbon atoms in both glucose and alanine. The rate of gluconeogenesis from alanine (mmol·h$^{-1}$·kg$^{-1}$) was then obtained as:

Gluconeogenesis = % glucose from alanine × Ra of $[^3\text{H}]_\text{glucose}$

Finally, the percentage of alanine converted into glucose was calculated by dividing the rate of gluconeogenesis from alanine by the rate of appearance of alanine [31].

**Statistical analysis**

Results are presented as means ± S.D. Changes in turnover between baseline and ATP infusion were tested for significance by the two-tailed Wilcoxon’s signed rank test. Results from independent groups were tested for significance of differences by the Mann–Whitney U-test. Correlations between variables were calculated as Spearman’s rank correlation coefficients. Results were considered statistically significant at $P < 0.05$.

**RESULTS**

**Study populations**

The characteristics of the study populations are shown in Table 1. A total of 12 NSCLC patients (nine males, three females) with a mean (± S.D.) age of 64 ± 13 years and a mean body weight of 72.7 ± 13.0 kg participated in the study. Mean weight loss was 7.1 ± 9.0%. Patients had received an average of 2.3 ± 2.1 previous ATP courses. Furthermore, 11 healthy subjects (three males, eight females; age 56 ± 12 years; body weight 79.5 ± 11 kg; no weight loss) and 13 NSCLC patients (10 males, three females; age 64 ± 14 years; body weight 67.8 ± 13.1 kg; weight loss 6.3 ± 9.9 kg) were included as control groups without ATP infusion.

**Glucose and alanine metabolism**

Baseline plasma glucose and alanine concentrations were 4.9 ± 0.8 mmol/l and 0.36 ± 0.02 mmol/l respectively, and these did not change during ATP infusion. When data were analysed for the ATP-treated patient group as a whole, turnover rates of glucose and alanine, and gluconeogenesis from alanine, during ATP infusion did not differ significantly from baseline. However, as shown in Figure 1, stratification for ATP dose revealed clear differences according to ATP dose. In patients with low-dose ATP infusion (37–50 µg·min$^{-1}$·kg$^{-1}$), no change was detected in glucose turnover (baseline, 0.58 ± 0.10 mmol·h$^{-1}$·kg$^{-1}$; during ATP infusion, 0.56 ± 0.13 mmol·h$^{-1}$·kg$^{-1}$) or gluconeogenesis from alanine (baseline, 0.34 ± 0.17 mmol·h$^{-1}$·kg$^{-1}$; during ATP infusion, 0.35 ± 0.24 mmol·h$^{-1}$·kg$^{-1}$). In contrast, in patients with high-dose ATP infusion (75 µg·min$^{-1}$·kg$^{-1}$), glucose turnover was 0.44 ± 0.13 mmol·h$^{-1}$·kg$^{-1}$ at
Table 1  Clinical details of NSCLC patients treated or not with ATP, and of healthy subjects
Values are means ± S.D.

<table>
<thead>
<tr>
<th>Cancer patients</th>
<th>Healthy subjects (no ATP)</th>
</tr>
</thead>
<tbody>
<tr>
<td>No ATP</td>
<td>ATP infusion</td>
</tr>
<tr>
<td>n</td>
<td>11</td>
</tr>
<tr>
<td>Sex (male/female)</td>
<td>3/8</td>
</tr>
<tr>
<td>Age (years)</td>
<td>56 ± 12</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>79.5 ± 11.0</td>
</tr>
<tr>
<td>Weight loss (%)</td>
<td>0 ± 0</td>
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<tr>
<td>Previous ATP courses</td>
<td>–</td>
</tr>
<tr>
<td>ATP dose (µmol·min⁻¹·kg⁻¹)</td>
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Figure 1  Whole-body rates of turnover of glucose and gluconeogenesis from alanine before and during (22–24 h) low-dose and high-dose ATP infusion

Low-dose ATP infusion (○) was at a rate of 37–50 µmol·min⁻¹·kg⁻¹ (n = 7), and high-dose infusion (●) was at a rate of 75 µmol·min⁻¹·kg⁻¹ (n = 5). Data are shown for individual subjects. Turnover rates were assessed using primed constant infusions of [6,6-H₂]glucose and [3-¹³C]alanine. Significant difference from baseline: * P < 0.05.

Baseline and 0.62 ± 0.07 mmol·h⁻¹·kg⁻¹ during ATP infusion (P = 0.04 for change). Gluconeogenesis from alanine amounted to 0.30 ± 0.16 mmol·h⁻¹·kg⁻¹ at baseline and to 0.37 ± 0.13 mmol·h⁻¹·kg⁻¹ during ATP infusion (P = 0.08). The change in glucose turnover from baseline was significantly correlated with the ATP dose (r = 0.58, P = 0.02). No such dose–effect relationship was demonstrated for gluconeogenesis from alanine.

Mean glucose turnover rates in ATP-treated and control NSCLC patients, and in healthy subjects, are shown in Figure 2. Glucose turnover rates in NSCLC patients during high-dose ATP infusion were similar to those in control (untreated) NSCLC patients, but significantly higher than those in healthy control subjects (P = 0.047). In contrast, baseline glucose turnover rates in high-dose ATP-treated NSCLC patients were significantly lower when compared with both low-dose ATP-treated and control NSCLC patients (P = 0.04 and P = 0.03 respectively), but were at a level similar to or even lower than those in healthy subjects.
The aim of the present pilot study was to explore the effects of intravenous ATP infusion on whole-body glucose turnover, alanine turnover and gluconeogenesis from alanine, as possible pathways contributing to the reported beneficial effects of ATP on body weight and body composition in patients with advanced lung cancer. The effect of ATP on gluconeogenesis from the amino acid alanine was studied because ATP has been shown to inhibit loss of skeletal muscle mass [23] and muscle strength [22]. Turnover measurements were performed 22–24 h after starting the ATP infusion, i.e. after ATP had reached plateau levels in erythrocytes [25]. All but one of the patients were studied during one of the subsequent ATP infusions.

During high-dose ATP infusion (75 μg·min⁻¹·kg⁻¹), whole-body glucose turnover increased by approx. 50%, whereas no change was shown during low-dose ATP infusion (37–50 μg·min⁻¹·kg⁻¹). An ATP-induced increase in gluconeogenesis from alanine explained only part of the increase in total hepatic glucose production. This would suggest that ATP may also stimulate gluconeogenesis from other substrates, as well as glycogenolysis. In vitro studies have shown that ATP administration stimulates gluconeogenesis from lactate [32,33], pyruvate [32,34] and glutamine [32,33,35,36]. Studies with isolated hepatocytes [37–39] and perfused rat liver [40,41] have shown that ATP stimulates glycogenolysis by activating glycogen phosphorylase. It is conceivable that high-dose ATP infusion evoked immediate stimulation of glycogenolysis in our patients, since turnover measurements were performed after an overnight fast of 10–12 h. Glycogen stores in healthy subjects have been reported to be depleted only after 36 h of fasting [42]; no data on glycogen stores in cancer patients are available.

The mechanisms responsible for increased glucose turnover and gluconeogenesis during 24 h of high-dose ATP infusion remain to be elucidated. These might include receptor-stimulating and catecholamine hormone-stimulating effects of ATP. Studies in isolated hepatocytes have shown that extracellular ATP induces phosphatidylinositol hydrolysis, mobilization of intracellular Ca²⁺ and influx of extracellular Ca²⁺ by stimulation of surface purinergic P₂ receptors [38,43], which are involved in the control of gluconeogenesis [44] and glycogenolysis [45]. Furthermore, ATP was shown to act as a co-transmitter of the catecholamine noradrenaline in the nervous system, and was suggested to modulate the release of other neurotransmitters [46]. Noradrenaline is an activator of gluconeogenesis [32,47] and glycogenolysis [47].

However, the above reasoning would only apply to the immediate effects of ATP infusion in NSCLC subjects. Notably, baseline glucose turnover rates in the present study were significantly lower in patients who had previously received high-dose ATP infusions when compared with patients who had received low-dose ATP or no ATP at all (controls). Since patients in the high-dose group had already undergone an average of two ATP courses before the present turnover measurements, our data would suggest that the previous ATP infusions had induced a decrease in whole-body glucose turnover on a longer term, which would be consistent with the observed inhibition of weight loss in our long-term clinical trial in NSCLC patients. This hypothesis is supported by the observation that baseline glucose turnover rates in patients who had already received one or more high-dose ATP infusions were similar to, or even lower than, those in control subjects.

In contrast, baseline gluconeogenesis from alanine did not differ significantly between the low- and high-dose ATP groups. This would be consistent with the finding in several experimental studies in vivo that the ATP degradation product adenosine inhibited gluconeogenesis from lactate [48–50], pyruvate [48,50] and glutamine [48,50], but not that from alanine [49,50]. In the present human study, direct effects of ATP on glucose turnover and gluconeogenesis cannot be separated from potential effects of adenosine.

In conclusion, the present study suggests that, despite a temporary dose-dependent increase in whole-body glucose turnover during high-dose ATP infusion in patients with advanced lung cancer, ATP seems to inhibit glucose turnover in the long term. This hypothesis is supported by the observation of reduced baseline glucose turnover rates (to levels similar to those in healthy cancer patients) in patients with high-dose ATP infusions, which would not differ significantly between the low- and high-dose ATP groups.

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