Glucose, but not glutamine, protects against spontaneous and anti-Fas antibody-induced apoptosis in human neutrophils

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ABSTRACT

Neutrophils are phagocytic cells of the innate immune system that use a combination of reactive oxygen species and anti-microbial toxins to kill and destroy ingested micro-organisms. Once they have performed their function, neutrophils die by apoptosis, which is important for the effective resolution of the inflammatory response. Both glucose and glutamine are important fuels for neutrophils, yet little has been done to investigate the comparative effects of glucose and glutamine on neutrophil apoptosis. We hypothesized that glucose and/or glutamine significantly alter rates of spontaneous and anti-Fas antibody-induced apoptosis of human neutrophils cultured ex vivo. Neutrophil apoptosis was reduced by increasing the extracellular concentration of glucose, but was unaffected by glutamine concentration. The protective effect of glucose appeared to correlate with the rate of glucose utilization. The addition of a competitive inhibitor of glycolysis, 2-deoxy-D-glucose (10 mM), attenuated the protective effect of 5.5 mM glucose, indicating that glucose metabolism is essential for its protective effect against apoptosis. There was a significant (P < 0.05) reduction in the intracellular ATP concentration of neutrophils incubated in the absence of extracellular glucose compared with cells incubated in the presence of 5.5 mM glucose. The protective effect of glucose against apoptosis may be mediated by maintenance of the intracellular ATP concentration.

INTRODUCTION

Polymorphonuclear granulocytes (neutrophils) are derived from stem cells and mature in the bone marrow. They constitute 50–60% of the total leucocytes and are the first cells to be recruited to a site of infection or injury [1]. The transmigration of neutrophils to a site of inflammation is associated with increased cellular activation and an increase in their half-life [2], resulting in their accumulation. Upon activation, neutrophil responses include adhesion to endothelial cells, migration through this cell layer into the inflammatory site (chemotaxis), and phagocytosis and killing of microbes [3,4]. Toxic metabolites of superoxide, such as hydrogen peroxide (H2O2), hypochlorous acid (HOCl) and hydroxyl radicals (OH•), are

Key words: apoptosis, anti-Fas antibody, glucose, glutamine, neutrophil.
Abbreviations: 2-DG, 2-deoxy-D-glucose; DMEM, Dulbecco’s modified Eagle’s medium; PI, propidium iodide.
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potent antimicrobial agents, which, in addition to elastase, collagenase, myeloperoxidase and lysozyme, can be released by the neutrophil to undertake its primary function of protecting the host. Once a neutrophil has performed its function, it dies by the process of apoptosis [5]. Apoptotic cells have the ability to be recognized and engulfed by phagocytes, facilitating the resolution of inflammatory responses and thereby limiting host tissue damage. Regulating neutrophil apoptosis has important implications for preventing the persistence of the inflammatory response. Disorders such as cystic fibrosis [6], acute respiratory distress syndrome [7], acute pancreatitis [8] and inflammatory bowel disease [9] have been associated with delayed neutrophil apoptosis, which leads to their persistence and associated damage of these organs due to inflammatory mediators such as reactive oxygen intermediates.

The relationship between nutrient metabolism and neutrophil activation remains unclear. In 1959, Sbarra and Karnovsky [10] reported an increase in glucose oxidation during phagocytosis, and glucose has long been believed to be the principle energy source of the neutrophil [11]. However, more recent reports now suggest that glutamine may be an important fuel for neutrophils and other phagocytes, especially where glucose use is restricted [12]. In fact, the rate of utilization of glutamine may be similar to that of glucose [13]. Neutrophils (and other immune cells, such as macrophages and lymphocytes) utilize both glucose and glutamine at high rates, but it appears that very little of the glucose or glutamine is oxidized via the tricarboxylic acid cycle [14]. Glucose is converted almost entirely into lactate, whereas glutamine is converted into glutamate, aspartate and lactate. The high rates of glutaminolysis and glycolysis in neutrophils provide precursors for biosynthetic pathways to support their high phagocytic and secretory activity [15].

As glucose and glutamine are important to neutrophil function, perturbations from the normal physiological concentrations of approx. 5 mM and 0.7 mM respectively could result in altered neutrophil function. The extra-cellular glucose concentration has been reported to alter phospholipase D activity and superoxide anion generation in neutrophils isolated from diabetic patients [16]. Decreases in plasma glutamine levels have been observed following surgery, trauma and burns [17–19], probably due to increased glutamine consumption by a number of tissues and cells of the immune system [20]. Depressed neutrophil function has been observed in such conditions [21,22]. Both ex vivo and in vitro studies have shown that depressed neutrophil bactericidal functions can be restored by addition of glutamine [23]. Several studies also report a decrease in the plasma concentration of glutamine along with a temporary suppression of immune function following prolonged exercise or over-training [24]. However, the findings of a recent study do not support a mechanistic role for glutamine in exercise-induced immune changes [25].

Despite the large number of reports describing the effects of nutrients on an array of functional parameters, the effects of nutrient availability on the rate of spontaneous and anti-Fas antibody-induced neutrophil apoptosis have not been rigorously investigated prior to the study reported here.

**MATERIALS AND METHODS**

**Reagents**

Dulbecco’s modified Eagle’s medium (DMEM) containing neither glucose nor glutamine was obtained from Gibco. Dextran T500 was purchased from Amersham Pharmacia Biotech A/B (Uppsala, Sweden). Lymphoprep was obtained from Nycomed (Oslo, Norway). A glutathione assay kit (Bioxytech GSH-400) was purchased from Oxis International Inc. (Portland, OR, U.S.A.). An ATP assay kit was purchased from Sigma Chemical Co. (Poole, Dorset, U.K.).

**Informed consent**

All human blood donors gave informed consent to inclusion in this study. They were not ingesting any form of medication at the time of blood donation.

**Isolation of human neutrophils**

Neutrophils were isolated by dextran sedimentation and centrifugation for 30 min at 400 g through Lymphoprep [26]. Isolated neutrophils were resuspended at a concentration of 1 × 10⁶ cells/ml in glucose- and glutamine-free DMEM supplemented with 10 % (v/v) fetal calf serum and 1 % penicillin/streptomycin solution. Immediately after isolation, neutrophil viability was always > 95 %, as assessed by Trypan Blue exclusion.

**Measurement of neutrophil apoptosis**

Neutrophil apoptosis was measured by analysis of the percentage of cells with hypodiploid DNA [27]. Neutrophils were cultured in DMEM supplemented with various concentrations of glucose (0, 1, 5.5 or 22 mM) and/or glutamine (0, 0.1, 0.2, 0.5, 1, 2 or 5 mM), and in the presence or absence of anti-Fas antibody (100 ng/ml), for 24 h at 37 °C in 5% CO₂. Cells (1 × 10⁶) were centrifuged at 200 g for 5 min, then resuspended in 400 μl of hypotonic propidium iodide (PI) solution (50 μg/ml PI, 3.4 mM sodium citrate, 1 mM Tris, 0.1 mM EDTA and 0.1 % Triton X-100) and stored in the dark at 4 °C before analysis using a Becton Dickinson FACStar Plus Flow Cytometer with laser excitation at 488 nm, using a 530/30 nm band pass filter to collect the PI fluorescence. A total of 10000 events were collected per sample, and cells were gated on the basis of forward (cell size) and side
Glucose protects against neutrophil apoptosis

Measurement of the rate of glucose utilization

Neutrophils were cultured in DMEM supplemented with various concentrations of glucose (0, 1, 5.5 or 22 mM) and/or glutamine (0, 0.1, 0.2, 0.5, 1, 2 or 5 mM). The glucose concentration of each medium was determined at the beginning of the incubation period using a commercially available glucose assay kit (Sigma). After 24 h of incubation, the neutrophil suspensions were centrifuged at 200 g for 5 min and the concentration of glucose in each medium supernatant was determined as before. The rate of glucose utilization was expressed as nmol · 24 h⁻¹ · 10⁸ cells⁻¹.

Measurement of the rate of glutamine utilization

Neutrophils were cultured in DMEM supplemented with various concentrations of glucose (0, 1, 5.5 or 22 mM) and/or glutamine (0, 0.1, 0.2, 0.5, 1, 2 or 5 mM). The concentration of glutamine in each medium at the beginning and end of the incubation period was measured using a protocol based on that described previously [29]. The amount of glutamine taken up by the neutrophils was obtained by subtracting the 24-h value from the 0-h value, taking spontaneous hydrolysis into account. The rate of glutamine utilization was expressed as nmol · 24 h⁻¹ · 10⁸ cells⁻¹.

Assay of cellular GSH

After incubation, 5 × 10⁶ neutrophils were resuspended in 500 µl of ice-cold metaphosphoric acid solution, mixed well, and centrifuged at 400 g at 4 °C for 10 min. The upper clear aqueous layer was collected and kept on ice. GSH levels were determined using a commercially available colorimetric assay (Bioxytech GSH-400 kit; Oxis International Inc.) based on the formation of a chromophoric thione in a reaction involving GSH.

Assay of cellular ATP

After incubation, approx. 6 × 10⁶ neutrophils were suspended in 1 ml of 12% trichloroacetic acid for 5 min at 4 °C. Intracellular ATP levels were determined using a commercially available colorimetric ATP assay kit (Sigma Chemical Co.) based on an enzymic reaction involving ATP that results in the oxidation of NADH to NAD⁺. The ATP concentration is proportional to the decrease in absorbance of NADH at 340 nm.

Statistical analysis

Data presented are means ± S.D. Statistical analyses were performed using Student’s t test, ANOVA or Spearman’s test as appropriate. A P value of < 0.05 was considered significant.

RESULTS

Effect of extracellular glucose and glutamine concentrations on neutrophil apoptosis

Immediately after isolation, the percentage of apoptotic neutrophils was 1.3 ± 0.9%. Percentage neutrophil apoptosis increased significantly to 24.3 ± 5.6% (P < 0.01) and 46.1 ± 12.4% (P < 0.01) after 24 h of culture in the absence and presence respectively of anti-Fas antibody. Increases in the extracellular concentration of glucose were found to reciprocally reduce the rates of spontaneous and anti-Fas antibody-induced neutrophil apoptosis. After 24 h of incubation in medium deficient in both glucose and glutamine, the percentage of spontaneous neutrophil apoptosis was 25.3 ± 5.7% (Table 1). This value was lowered to 19.4 ± 4.3% (P < 0.05), 14.3 ± 3.5% (P < 0.01) and 14.0 ± 3.8% (P < 0.01) for neutrophils incubated in medium containing 1, 5.5 and 22 mM glucose respectively (Table 1). The percentage of neutrophils undergoing anti-Fas antibody-induced apoptosis was 46.6 ± 7.5% after 24 h of incubation in medium deficient in both glucose and glutamine. This value was lowered to 36.0 ± 6.7% (P < 0.05), 26.6 ± 4.7% (P < 0.01) and 23.5 ± 4.3% (P < 0.01) for neutrophils incubated in medium containing 1, 5.5 and 22 mM glucose respectively (Table 2).

There was no change in the rate of either spontaneous or Fas antibody-induced apoptosis as the concentration of glutamine in the incubation medium was increased from 0 to 5 mM (concentrations used were 0, 0.1, 0.2, 0.5, 1, 2 or 5 mM).

(continued)
### Table 1  Effects of various extracellular concentrations of glucose and glutamine on the rate of spontaneous neutrophil apoptosis in vitro

Neutrophils were incubated with various concentrations of glucose (0, 1, 5 or 22 mM) and glutamine (0, 0.1, 0.2, 0.5, 1, 2 or 5 mM) for 24 h. After this time, the percentage of apoptotic neutrophils was assessed by measuring the percentage of cells with hypodiploid DNA following PI staining. Values are mean percentages of apoptotic neutrophils ± S.D. (n = 6). Statistical analysis was performed by ANOVA using Dunnett’s post-hoc test. Values within a row with an alphabetic superscript not containing a common letter are significantly different (P < 0.05).

<table>
<thead>
<tr>
<th>[Glutamine] (mM)</th>
<th>[Glucose] (mM) …</th>
<th>Apoptosis (%)</th>
</tr>
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<tbody>
<tr>
<td>0</td>
<td>0</td>
<td>25.3 ± 5.7a</td>
</tr>
<tr>
<td>0.1</td>
<td>0.5</td>
<td>24.3 ± 4.2b</td>
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<td>23.5 ± 4.7a</td>
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<td>0.5</td>
<td>24.6 ± 3.9b</td>
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<td>23.0 ± 5.0a</td>
</tr>
<tr>
<td>2</td>
<td>0.5</td>
<td>22.3 ± 5.5a</td>
</tr>
<tr>
<td>5</td>
<td>0.5</td>
<td>22.9 ± 3.8a</td>
</tr>
</tbody>
</table>

### Table 2  Effects of various concentrations of glucose and glutamine on the rate of anti-Fas antibody-induced neutrophil apoptosis in vitro

Neutrophils were incubated with various concentrations of glucose (0, 1, 5 or 22 mM) and glutamine (0, 0.1, 0.2, 0.5, 1, 2 or 5 mM), and in the presence of 100 ng/ml anti-Fas antibody, for 24 h. After this time, the percentage of apoptotic neutrophils was assessed. Values shown are mean percentages of apoptotic neutrophils ± S.D. (n = 5). Statistical analysis was performed by ANOVA using Dunnett’s post-hoc test. Values within a row with an alphabetic superscript not containing a common letter are significantly different (P < 0.05).

<table>
<thead>
<tr>
<th>[Glutamine] (mM)</th>
<th>[Glucose] (mM) …</th>
<th>Apoptosis (%)</th>
</tr>
</thead>
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<tr>
<td>0</td>
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<tr>
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<td>0.5</td>
<td>42.9 ± 8.2c</td>
</tr>
</tbody>
</table>

### Table 3  Effects of extracellular glucose and glutamine concentrations on the rate of glucose utilization by human neutrophils

Human neutrophils (3 × 10⁶/ml of DMEM) were incubated at various concentrations of extracellular glucose and glutamine, and in the presence or absence of 100 ng/ml anti-Fas antibody. Values represent means ± S.D. from six separate experiments. Statistical analysis was performed by ANOVA using Dunnett’s post-hoc test. Values within a row with an alphabetic superscript not containing a common letter are significantly different (P < 0.05).

<table>
<thead>
<tr>
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<th>[Glucose] (mM) …</th>
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<td>205.3 ± 38.2a</td>
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<td>2</td>
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<td>0.5</td>
<td>214.1 ± 39.4a</td>
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<td>206.1 ± 35.4a</td>
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<td>286.9 ± 38.3a</td>
</tr>
<tr>
<td>5</td>
<td>0.5</td>
<td>326.7 ± 42.0a</td>
</tr>
</tbody>
</table>

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Glucose protects against neutrophil apoptosis

Figure 1 Correlation between the rate of glucose utilization and percentage spontaneous and anti-Fas antibody-induced human neutrophil apoptosis

Human neutrophils (3 × 10^6/ml) were incubated at various concentrations of glucose (0, 1, 5.5 or 22 mM), with 1 mM glutamine, in the presence or absence of anti-Fas antibody (100 ng/ml) for 24 h. The rate of glucose uptake by neutrophils and the percentage of apoptotic cells were determined and plotted. A total of six blood donors were used for each experiment. Each data point shown is the mean value of three independent experiments. Using Spearman’s test, statistically significant correlations between the rate of glucose uptake and the rates of spontaneous (P < 0.01) and anti-Fas antibody-induced (P < 0.01) apoptosis were observed.

![Graph showing correlation between glucose utilization and apoptosis](image)

1.0, 2.0 and 5.0 mM; Tables 1 and 2). Additionally, glutamine had no effect on apoptosis at glucose concentrations of 1, 5.5 and 22 mM (Tables 1 and 2).

Effect of extracellular glucose and glutamine concentrations on their rates of utilization

Cells undergoing apoptosis have been reported to exhibit alterations in their rate of glucose transport [30–34]. Therefore the effects of various concentrations of extracellular glucose (0, 0.5, 1, 5.5 and 22 mM) and glutamine (0, 0.1, 0.2, 0.5, 1 and 2 mM) on the rates of utilization of these metabolites were investigated.

The rate of glucose utilization by neutrophils appeared to be dependent on the concentration of glucose present in the medium (Table 3). The rate of glucose utilization was not dependent either on the extracellular glucose concentration (Table 3) or on the presence or absence of anti-Fas antibody (results not shown). A negative and statistically significant correlation (P < 0.01) was observed between the rate of glucose utilization and the percentage of neutrophils undergoing spontaneous or Fas antibody-induced apoptosis (Figure 1). The apparent K_m for glucose utilization was determined as 0.62 mM, and the apparent V_max was 352 nmol · 24 h⁻¹ · 10^6 cells⁻¹ (Figure 2).

The rate of glutamine utilization by neutrophils was dependent on the concentration of extracellular glutamine in the medium (Table 4). The rate of glutamine utilization was additionally dependent upon the extracellular glucose concentration (Table 4). Glutamine utilization was not altered by the presence or absence of anti-Fas antibody (results not shown). The apparent K_m and V_max values for glutamine utilization were determined to be 1.29 mM and 333.8 nmol · 24 h⁻¹ · 10^6 cells⁻¹ respectively for neutrophils incubated in the absence of extracellular glucose. Increasing the extracellular concentration of glucose from 0 to 1 or 5.5 mM decreased the apparent V_max of glutamine utilization by 27% (to 243.9 nmol · 24 h⁻¹ · 10^6 cells⁻¹) and 57% (to 142.6 nmol · min⁻¹ · mg⁻¹ protein) respectively (Figure 3).

Effects of 2-deoxy-D-glucose (2-DG) on neutrophil apoptosis

In order to determine whether the protective effect of glucose against neutrophil apoptosis was dependent on a direct effect of glucose or on its metabolism, the effect of
Table 4  In vitro rate of neutrophil glutamine utilization at various extracellular concentrations of glucose and glutamine

Neutrophils were incubated with various concentrations of glucose (0, 1, 5.5 or 22 mM) and glutamine (0, 0.1, 0.5, 1 or 2 mM) for 24 h. Values represent means ± S.D. from six separate experiments. Statistical analysis was performed by ANOVA using Dunnett’s post-hoc test. Values within a column with an alphabetic superscript not containing a common letter are significantly different (P < 0.05). Values within a row indicated by a symbolic superscript (*, †, ‡) are significantly different (P < 0.05) from the value at 0 mM glucose, and values indicated by different symbolic superscripts are significantly different from each other (P < 0.05).

<table>
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<tr>
<th>[Glutamine] (mM)</th>
<th>[Glucose] (mM)</th>
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<th>22</th>
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<tr>
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<td>116.9 ± 29.3c</td>
<td>60.1 ± 18.2*</td>
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<td>2</td>
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<td>141.7 ± 43.6*</td>
<td>83.9 ± 8.8†</td>
<td>17.6 ± 13.2†</td>
<td></td>
</tr>
</tbody>
</table>

Figure 3  Determination of kinetic parameters with respect to glutamine utilization by human neutrophils at different glucose concentrations using a Lineweaver–Burk plot

Human neutrophils were incubated at various concentrations of glutamine (0, 0.1, 0.5, 1 or 2 mM) and glucose (0, 1, 5.5 or 22 mM) for 24 h, and the rate of glutamine utilization was measured as described in the Materials and methods section. Each data point is the mean of values obtained from five neutrophil donors.

2-DG (a competitive inhibitor of glycolysis) was investigated. 2-DG (10 mM) completely inhibited the protective effect of 5.5 mM glucose against both spontaneous and anti-Fas antibody-induced apoptosis, and caused approx. 3.5-fold increases in the rates of spontaneous and anti-Fas antibody-induced apoptosis compared with the rates observed in the absence of 2-DG (Figure 4; medium 3 vs 2). When the concentration of glucose in the medium was increased to 22 mM in the presence of 10 mM 2-DG, the protective effect of glucose against both spontaneous and anti-Fas antibody-induced apoptosis was completely restored, such that the rates of spontaneous and anti-Fas antibody-induced apoptosis were comparable with those...
Glucose protects against neutrophil apoptosis

Figure 5  Effects of extracellular glucose and glutamine on the intracellular GSH concentration in human neutrophils

Neutrophils (3 x 10^6/ml) were incubated for 24 h in the presence or absence of glucose (5.5 mM), glutamine (2 mM), anti-Fas antibody (100 ng/ml). The GSH content of the neutrophils was then measured. Values shown are means ± S.D. (n = 6). *P < 0.05 compared with value in the absence of anti-Fas antibody (Student’s t test).

Effect of extracellular glucose concentration on intracellular GSH levels

Intracellular glutathione (reduced form; GSH) has been shown to be an important regulator of human neutrophil apoptosis [35]. The glucose concentration has been shown previously to impact upon intracellular GSH levels in several cell types [36]; therefore the effect of extracellular glucose on intracellular GSH levels in neutrophils was investigated. The extracellular glucose concentration did not alter neutrophil intracellular GSH levels. In addition, intracellular GSH was not altered significantly by the presence of anti-Fas antibody, except when the neutrophils were incubated in medium containing neither glucose nor glutamine (Figure 5).

Effect of extracellular glucose concentration on intracellular ATP content

The ATP concentration is important for the process of cellular apoptosis, via provision of energy for dismantling of cellular structure. It is not clear, however, if a perturbation of the cellular ATP concentration triggers apoptosis directly [37]. The effect of glucose on the ATP content of neutrophils was examined in the present study.

The ATP content of neutrophils after a 24 h incubation in the presence of 5.5 mM glucose was approx. 17.5 nmol/mg protein. This was similar to a value of 19.3 ± 1.9 nmol/mg protein determined in freshly isolated neutrophils. The extracellular glucose concentration appeared to be positively correlated with the intracellular ATP concentration (Figure 6). The intracellular ATP concentration was significantly higher at 5.5 or 22 mM glucose (Figure 4; medium 4 vs 2).

Effect of extracellular glucose concentration on intracellular ATP content

Human neutrophils (3 x 10^6/ml) were incubated for 24 h in the presence of various extracellular concentrations of glucose (0, 1, 5.5 or 22 mM) and 2 mM glutamine, and in the presence or absence of anti-Fas antibody (100 ng/ml). The intracellular concentration of ATP was then determined. Values are means ± S.D. (n = 6). Statistical analysis was performed by ANOVA using Dunnett’s post-hoc test; *P < 0.05 compared with ATP values measured at 0 mM glucose.

Figure 6  Effect of extracellular glucose on intracellular ATP concentration in human neutrophils in the presence or absence of anti-Fas antibody

Figure 7  Correlation between the percentage of apoptotic cells and the mean intracellular ATP content of human neutrophils

Human neutrophils were incubated for 24 h in the presence of 0, 1, 5.5 or 22 mM glucose, and in the presence or absence of anti-Fas antibody. Each data point represents the mean of values obtained from four separate experiments for each neutrophil donor; five donors were used. Significant correlations were observed between the neutrophil intracellular ATP content and the percentage of neutrophils undergoing spontaneous (P < 0.05) and anti-Fas antibody-induced (P < 0.05) apoptosis (Spearman’s test).
glucose than at 0 mM glucose for cells undergoing both spontaneous and anti-Fas antibody-induced apoptosis (Figure 6; \( P < 0.05 \) in each case). Cells incubated in the presence of anti-Fas antibody tended to have a lower intracellular concentration of ATP than cells incubated without antibody, but these differences were not significant at any of the extracellular glucose concentrations tested (Figure 6).

A negative and statistically significant correlation \(( P < 0.05)\) was observed between the percentage of apoptotic (spontaneous and anti-Fas antibody-induced) neutrophils and the intracellular ATP concentration (Figure 7).

**DISCUSSION**

The results presented in this paper clearly demonstrate that the concentration of glucose in the *in vitro* environment of incubated human neutrophils impacts upon their rate of apoptosis. Spontaneous and anti-Fas antibody-induced apoptosis was inhibited in a dose-dependent manner in the presence of 1, 5.5 or 22 mM glucose after a 24 h incubation, as assessed by DNA fragmentation (Tables 1 and 2) and cell morphology (results not shown).

Our data may indicate that there is an increase in spontaneous neutrophil apoptosis under hypoglycaemic conditions compared with normoglycaemic conditions. Thus diabetic patients with temporary insulin-induced hypoglycaemia or hypoglycaemic endurance athletes may experience decreased efficiency of neutrophil-dependent immune responses.

At a high extracellular concentration of glucose (22 mM), the rates of both spontaneous and anti-Fas antibody-induced apoptosis were the same as those observed at 5.5 mM. Thus it appears that the normal physiological concentration of glucose is sufficient to confer maximal protection against both spontaneous and anti-Fas antibody-induced apoptosis.

The protective effect of glucose against neutrophil apoptosis correlated significantly with the rate of glucose utilization (Figure 1). This finding suggests that extracellular glucose may affect neutrophil apoptosis only after it has been transported into the cells. Evidence exists to support a link between the rate of glucose uptake and the rate of apoptosis in several cell types. Uptake and glycolytic metabolism of extracellular glucose has been shown to protect cardiac myocytes from hypoxic injury-induced apoptosis [30]. Apoptosis is thought to be regulated by the rate of glucose transport into an interleukin 3-dependent cell line [31] and rat hepatoma cells [32]. In addition, CD95 (Fas)-induced apoptosis in a human leukemic cell line resulted in the loss of glucose transporter function [33]. Ceramide, a mediator of CD95- and tumour necrosis factor \( \alpha \)-induced apoptosis, has been shown to reduce glucose transport into human neutrophils and leukaemic cell lines [34].

The apparent \( K_m \) value for glucose utilization by neutrophils determined in the work presented here was 0.67 mM. This suggests that a GLUT1 transporter may be involved in glucose uptake by neutrophils, since a \( K_m \) of 0.67 mM is close to that reported for GLUT1 transporters in other cell types [38]. The protective effect of 5.5 mM extracellular glucose against spontaneous and anti-Fas antibody-induced apoptosis was lost upon the addition of 10 mM 2-DG (a competitive inhibitor of glycolysis), possibly due to reduced glycolytic flux. This suggests that the protective effect against neutrophil apoptosis may not be a direct effect of glucose, but instead may be dependent on the production of a metabolite. Increasing the glucose concentration to 22 mM restored the protective effect even in the presence of 2-DG. This may be due to attenuation of the inhibitory effect of 2-DG on glucose metabolism, since 2-DG only inhibits glucose metabolism significantly when present at a concentration similar to or greater than that of glucose [39]. The rate of apoptosis was higher for neutrophils incubated in the presence of 5.5 mM glucose and 10 mM 2-DG than for control cells incubated in the absence of both glucose and 2-DG (Figure 4; medium 3 vs 1). This finding may be explained by the possibility that some glycolytic activity may occur in control cells due to the presence of intracellular glucose, whereas 10 mM 2-DG may completely inhibit glycolysis even for cells incubated in the presence of 5.5 mM glucose.

Despite the belief that glutamine is important for neutrophil function [40], the findings of the work presented here suggest that the extracellular concentration of glutamine does not affect the *in vitro* rate of either spontaneous or anti-Fas antibody-induced apoptosis of human neutrophils. The absence of an effect of glutamine on neutrophil apoptotic rates was evident even when glucose was absent from the incubation medium. This finding is in agreement with that reported by Furukawa et al. [41], who found that neutrophil apoptosis was similar at extracellular glutamine concentrations of 0, 0.5, 1 and 2 mM. This absence of an effect of glutamine suggests that, under conditions of glutamine depletion *in vivo*, such as those seen post-operatively, the associated impairment of immune function is not due to an impact of glutamine concentration on neutrophil apoptosis.

Despite the absence of an effect of extracellular glutamine on neutrophil apoptosis *in vitro*, the potential importance of glutamine to the neutrophil is indicated by its high rate of utilization, which was determined in the present work to be \( 83.9 \pm 8.8 \text{ nmol} \cdot 24 \text{ h}^{-1} \cdot 10^6 \text{ cells}^{-1} \) in the presence of 5.5 mM glucose and 2 mM glutamine (Table 4). The rate of glutamine utilization was dependent upon the concentration of extracellular glutamine, with apparent \( K_m \) and \( V_{max} \) values of 1.29 mM and 142.6 nmol \cdot 24 h^{-1} \cdot 10^6 cells^{-1} \approx 2.5 \text{ nmol} \cdot \text{min}^{-1} \cdot 10^6 \text{ cells}^{-1} \).
addition, Low et al. [43] reported
Extracellular glucose did not affect the apparent when the extracellular glucose concentration was reduced
respectively.
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and 0.05
Ramaharobandro et al. [45] reported a
V
max
glucose concentration. The apparent utilization was also dependent on the extracellular
extracellular glutamine, the apparent V
max
hypothesis that glutamine may be of particular im-
glutamine transport calculated here are similar to those reported for cells of the brain and liver supports the hypothesis that glutamine may be of particular importance to critical cellular functions, such as superoxide production and phagocytosis [23].

In addition to being dependent on the concentration of extracellular glutamine, the apparent V
max
of glutamine utilization was also dependent on the extracellular glucose concentration. The apparent V
max
increased by 71 % (from 142.6 to 243.9 nmol · min⁻¹ · mg of protein⁻¹ (K
m
0.30 mM), whereas Ramaharobandro et al. [45] reported a V
max
for sodium-independent glutamine transport by rat astrocytes of 2.1 nmol · min⁻¹ · mg of protein⁻¹ (K
m
0.21 mM). In addition, Low et al. [43] reported V
max
values for sodium-dependent and sodium-independent rates of glutamine transport across rat liver sinusoidal membranes of 5.30 ± 0.40 nmol · min⁻¹ · mg⁻¹ (K
m
1.20 ± 0.14 mM) and 0.05 ± 0.01 nmol · min⁻¹ · mg⁻¹ (K
m
0.014 mM) respectively.

The fact that the K
m
and V
max
values for neutrophil glutamine transport calculated here are similar to those reported for cells of the brain and liver supports the hypothesis that glutamine may be of particular importance to critical cellular functions, such as superoxide production and phagocytosis [23].

In conclusion, the
V
max
of glutamine utilization was dependent upon the extracellular concentration of glucose, (Figure 6). The ATP concentration of a cell is an important determinant of survival or death [37]. Glucose metabolism is one major source of ATP for a cell. The present study has reported that the intracellular neutrophil ATP concentration after 24 h of incubation in the presence of 5.5 mM glucose was ~15 nmol/mg protein (approximately twice the ATP content of macrophages, ~ 7 nmol/mg protein [40]). The intracellular ATP concentration was dependent upon the extracellular concentration of glucose (Figure 6). The ATP concentration of neutrophils incubated in the absence of glucose (or in the presence of an anti-Fas antibody) was 83 % of that of neutrophils incubated in the presence of 5.5 or 22 mM glucose (P < 0.05) (Figure 6).

Despite the fact that ATP is required for apoptosis to occur, ATP depletion is thought to be an important stimulus to the apoptotic cascade. It is known that the ATP concentration must fall substantially before apoptosis is triggered directly by this mechanism. For example, the concentration of ATP was reduced by 90–95 % in rat islet cells incubated in serum from patients with Type I diabetes before cell death occurred by apoptosis [47], while apoptosis was induced in insulin-producing pituitary cells overexpressing GLUT2 and glucokinase after exposure to 20 mM glucose, a condition which also reduced the ATP concentration by approx. 80 % [48]. The latter paper also reported a fall in ATP concentration of approx. 40 % in cells overexpressing glucokinase only, after exposure to 20 mM glucose, a condition that did not result in apoptosis of the cells. In the present study, intracellular ATP did not fall below 70 % of the pre-incubation value following incubation for 24 h in the absence of both glucose and glutamine. This may indicate that apoptosis is triggered by a number of key pathways in the neutrophil, one of which may involve a decrease in ATP concentration. The decrease in ATP concentration reported in the present paper may correlate with a decrease in the rate of production of ATP by oxidative phosphorylation, due to mitochondrial damage. The protective effect of glucose against spontaneous and anti-Fas antibody-induced neutrophil apoptosis may be mediated via a mechanism involving maintenance of the rate of intracellular ATP generation, due to a stabilizing effect on mitochondrial function. This hypothesis is supported by the fact that a significant and negative correlation was found between the intracellular ATP concentration and the rates of spontaneous and anti-Fas antibody-induced apoptosis (Figure 7). Fas-induced apoptosis is initiated by ATP-independent pathways [49], but the cellular ATP concentration may be indirectly correlated with levels of apoptosis in Fas-exposed cells.

In conclusion, the in vitro rates of spontaneous and anti-Fas antibody-induced neutrophil apoptosis are dependent upon the extracellular concentration of glucose, but not that of glutamine. The rate of glucose utilization by neutrophils was correlated negatively with rates of neutrophil apoptosis. The effect of glucose appeared to be independent of intracellular GSH, but may be due in part to maintenance of the intracellular ATP concen-
tration as a result of stabilization of mitochondrial function. It is also possible that the concentration of ATP is essential for key interactions regulating apoptosis, perhaps between caspases and their intracellular regulatory proteins.

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Glucose protects against neutrophil apoptosis


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