The potential effect of vanadium compounds on glucose-6-phosphatase†

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Type 2 diabetes is a major chronic health condition in which hyperglycaemia has significant impact on morbidity and mortality, and its ever-increasing incidence has made the production of therapeutic agents for type 2 diabetes more necessary. Vanadium compounds are known to control hyperglycaemia but the exact focus of where they work is a matter of debate. A proposed mechanism of action is that it inhibits glucose-6-phosphatase, a key enzyme in the development of insulin resistance and thus type 2 diabetes. This paper looks at the inhibitory effects of vanadium salts on glucose-6-phosphatase and also studies the mechanism of inhibition, the hypothesis being that the two vanadium compounds, vanadyl sulphate (VOSO₄) and vanadyl acetylacetonate (Vace) will inhibit glucose-6-phosphatase. This was achieved by using a proof of principle study by extracting glucose-6-phosphatase from bovine liver microsomes using differential centrifugation, and then the enzyme was assayed in the presence and absence of vanadium compounds. The study found that vanadyl compounds inhibit glucose-6-phosphatase, as mean specific enzyme activity was calculated which showed that VOSO₄ at a concentration of 48 µM inhibited glucose-6-phosphatase activity by 36.9% (P < 0.0003) and Vace at a concentration of 200 µM inhibited glucose-6-phosphatase activity by 50% (P < 0.0001) similar to findings in the previous research. However, this study also found using Lineweaver–Burk plot analysis that VOSO₄ is a competitive inhibitor of glucose-6-phosphatase and Vace is a mixed: non-competitive and uncompetitive inhibitors of glucose-6-phosphatase. The mechanism of inhibitory action of these vanadyl compounds had not been reported previously. The difference in the concentration of inhibitor required may be due to the type of inhibition. This supports the hypothesis to some extent as the results were found to be statistically significant; however, further data will be required to clarify these findings.

Keywords: glucose-6-phosphatase, vanadyl sulphate, vanadyl acetylacetonate, type 2 diabetes, inhibition, therapeutics

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Introduction

Diabetes mellitus, a disorder of blood glucose regulation, is known to occur in over 2.6 million people in the UK, and over half a million of people are unaware that they have this condition (NICE, 2008). This generic term encompasses two pathogenically different conditions, Type 1 and Type 2 diabetes. Type 1 diabetes is known to be auto-immune mediated, where the body attacks its own β-cells within the pancreas; these cells are responsible for the production of insulin required for blood glucose regulation. Type 2 diabetes is the more common type and will be the focus of this paper.

According to the National Institute for Health and Clinical Excellence (NICE, 2008) direct mortality attributable to type 2 diabetes is 4.2% in men and 7.7% in women within the UK. It suggests that a 60-year old man without any other arterial disease is expected to lose 8–10 years of his life without proper management. Its burden on the economy comprises its direct cost to the National Health Service due to managing it, indirect...
effect on the economy through early mortality and lost productivity and impact of this debilitating condition on patients and families, which places it on high priority to manage this condition effectively. NICE (2008) states that Type 2 diabetes occurs due to impaired sensitivity of insulin within the body plus inability by the pancreas to secrete insulin to compensate for this. Another characteristic found in type 2 diabetic patients is obesity, which has led to the understanding of a syndrome, known as metabolic syndrome. The cluster of abnormalities found within this population includes hyperglycaemia, hyperinsulinaemia, dyslipidaemia and hypertension. These are thought to be due to a genetic defect resulting in insulin resistance, a phenomenon which is exacerbated by obesity. If the pancreas cannot compensate for the apparent reduction in insulin then type 2 diabetes results. The mechanism by which these result remains in question (German and Masharani, 2007).

Insulin resistance was first studied by Himsworth in the 1930s who introduced the first standardized approach to quantifying insulin sensitivity in vivo (Kim, 2011). He carried out two oral glucose tolerance tests, one with and one without exogenous insulin in diabetics, and showed the insensitivity of this population to insulin. Plasma measurement of insulin was later made possible by radioimmunoassay for insulin in 1960s. Yalow and Berson (1960) demonstrated a delayed response to an oral glucose challenge in diabetic patients. The hyperglycaemia present following the glucose challenge proved the insensitivity towards insulin.

With this knowledge, the treatment of type 2 diabetes has been to regulate the body’s response to insulin and also to increase the production of insulin to compensate for this perceived lack of insulin. Table 1 shows the current agents used to control type 2 diabetes and how resistance and secretion are the recognized defects.

The mechanism of insulin resistance has been widely debated. A genetic component has been accepted as shown in Pima Indians where it is estimated that 30% of the variance in insulin insensitivity can be accounted by familial clustering (German and Masharani, 2007). Many genes have been implicated including the impaired activities of key enzymes involved in tissue glucose metabolism in insulin-responding tissues. One of these key enzymes is glucose-6-phosphatase, found within the liver and kidney cortex and involved in the final steps of gluconeogenesis, the production of glucose from non-carbohydrate carbon substances and glycogenolysis, where it completes the transformation to glucose from glucose-6-phosphate, so that it can be utilized within the body. As shown in Fig. 1, it hydrolyses the intermediate, glucose-6-phosphate, resulting in glucose and a free phosphate.

This enzyme is a target of insulin action, where it is inhibited in states of hyperglycaemia to prevent the production of glucose. In type 2 diabetes the resistance of liver to insulin
leads to uncontrolled gluconeogenesis. Clore, Stillman and Sugerman (2000) have shown in type 2 diabetes that there is an overexpression of glucose-6-phosphatase, leading to increased endogenous glucose production. Additionally, Cori and Cori (1952) demonstrated that deficiency of this enzyme is responsible for glycogen storage disease type 1, which leads to profound hypoglycaemia indicating its importance in plasma glucose homeostasis. Arion et al. (1972) showed this enzyme is found within the endoplasmic reticulum and its functionality was lost when extraction attempts were made, it requires an intact microsome for the enzyme to function which initially provided difficulties in studying this enzyme system (van Schaftingen and Gerin, 2002). It was found to catalyse specifically the hydrolysis of glucose-6-phosphate. The enzyme is 10 times more efficient in hydrolysing glucose-6-phosphate than mannose-6-phosphate and Nordlie and Arion (1964) showed it is capable of hydrolysing other substrates other than sugar phosphates such as pyrophosphate and carbamoyl phosphate. Due to its reaction mechanism shown in Fig. 2, Luecke and Nordlie (1970) demonstrated that glucose-6-phosphatase also has phosphotransferase activity, being able to synthesize glucose-6-phosphate from donors which includes glucose.

Due to the role of glucose-6-phosphatase in plasma glucose homeostasis, it gives inhibitors of phospho-transferases the ability to control hyperglycaemia, and though there are many treatments for type 2 diabetes, as listed in Table 1, there is potential for improvement in glycaemic control, thus phospho-transferase inhibitors are potential therapeutic agents. Lindquist, Lynn and Lienhard (1973) found vanadate to be a potent inhibitor of phospho-transferases and following this it was also found to inhibit various essential enzyme systems such as Na and K ATPases (Cantley et al., 1978), Ca-ATPase (O’Neal, Rhoads and Racker 1979), dynein ATPase (Kobayashi et al., 1978) and alkaline phosphatase (Lopez, Stevens and Lindquist, 1976).

Vanadium is a member of group VB of the periodic table. Two common salts of vanadium, vanadyl sulphate (VOSO₄) and vanadyl acetylacetonate (Vace), are shown in Figs 3 and 4, respectively. Although most food contains low amounts of vanadium (<1 ng/g), food is the major source of exposure to vanadium for the general population (Barceloux, 1999).

Cam, Brownsey, and McNeill (2000) demonstrated blood glucose concentration in insulin-dependent diabetic rats and humans was almost normalized after the administration of vanadium compounds. These effects on glucose metabolism are consistent with the observed lowering of blood glucose level of diabetic rats into which vanadate and vanadyl derivatives have been orally administered (Bevan et al., 1995). Tsiani et al. (1998) showed the mechanism by which vanadium salts reduce hyperglycaemia and improved insulin action is by increasing the glucose transporters activity via insulin receptor substrates 1 and 2 (IRS1/2), phosphatidylinositol 3-kinase (PI 3-kinase). Vanadium has also been found to activate serine/threonine kinases involved in intracellular insulin signalling at sites distal to the insulin receptor, thereby preventing protein dephosphorylation through inhibition of tyrosine phosphatases (Krentz, 2008).

![Figure 2](https://example.com/figure2.png)  
**Figure 2.** Reaction mechanism of glucose 6 phosphatase. In the presence of an adequate phosphate donor, the enzyme (E) forms a phosphoenzyme, which can be hydrolysed or can serve to phosphorylate another substrate. Under physiological conditions, only glucose-6-phosphatase serves as a substrate and the phosphoenzyme is hydrolysed. Source: Schaftingen and Gerin (2002).

![Figure 3](https://example.com/figure3.png)  
**Figure 3.** Molecular structure of VOSO₄. Vanadyl(IV) sulphate (VOSO₄) is a well-known inorganic compound of vanadium. This blue solid is one of the most common sources of vanadium in the laboratory, reflecting its high stability. It features the vanadyl ion, VO₂⁺.

![Figure 4](https://example.com/figure4.png)  
**Figure 4.** Molecular structure of Vace (Source: Google Images). Vace (Vace) is the chemical compound with the formula VO(C₅H₇O₂)₃. This blue–green coordination complex consists of the vanadyl group, VO₂⁺, bound to two acetylacetonate ions, acac−. Like other charge-neutral acetylacetonates, this complex is soluble in organic solvents.
As well as controlling hyperglycaemia, vanadium has also been shown to decrease triglyceride levels in the plasma, which is an important risk factor for the development of major chronic health conditions such as hypertension and coronary heart disease. Manninen et al. (1992) showed that the initial plasma triglyceride values of diabetic rats were markedly higher ($P < 0.05$) than those of non-diabetic rats. In diabetic rats groups receiving physiological saline (diabetic control), the plasma triglyceride levels remained at the initial higher triglyceride levels. After treatment with Vace, the plasma triglyceride levels in diabetic rats gradually decreased to the initial triglyceride values of non-diabetic rats. Similar to the triglyceride, the initial values of plasma cholesterol levels in diabetic rats were evidently higher ($P < 0.05$) than those of non-diabetic rats, and after the treatment with Vace, the high plasma cholesterol levels in diabetic rats gradually reversed to a normal range.

Fewer papers, including Kiersztan et al. (2004), have studied the effect of vanadium salts on glucose-6-phosphatase and have found it has an inhibitory effect; however, they have not commented on the mechanism of inhibition. There are three main mechanisms of inhibition, which are competitive, non-competitive, and uncompetitive, illustrated in Fig. 5a–c: (a) shows the competitive inhibition model, (b) the non-competitive inhibition model and (c) the uncompetitive inhibition model.

The aim of the current study is to verify the inhibition of glucose-6-phosphatase by vanadium compounds. It has been shown that vanadium sulphate exerts its effect at a lower concentration than Vace. This may be explained in different types of inhibition. To investigate this, a proof of principle study will be made using bovine liver microsome, which would be isolated using the modification of the method used by Woodward (2008), based on that of Ernster, Siekevitz and Palade (1962) and Hamilton et al. (1999). Glucose-6-phosphatase can be assayed using a modification to the method stated by Woodward (2008), Tauskky and Shorr (1953) and Nordlie and Arion (1966) and the tissue protein will be determined by a Lowry assay (Lowry et al., 1951). Singh, Nordlie and Jorgenson (1981) found sodium vanadate to be a competitive inhibitor and seemed to generalize it for all vanadium compounds. No data could be found on the mechanism of action of VOSO$_4$ and Vace on glucose-6-phosphatase. Therefore, the proposed hypothesis is that VOSO$_4$ and Vace will competitively inhibit glucose-6-phosphatase.

**Materials and Methods**

All materials and reagents were provided by Staffordshire University and chemicals were purchased from Sigma-Aldrich Co. (UK). Bovine liver samples were supplied from a local abattoir.

**Liver microsome isolation**

Liver tissue samples of 80–90 g were cut from whole bovine liver, delivered within ~2–4 h of animal death and kept on ice. Tissue samples were minced using a scalpel after rinsing with ice-cold 0.25 M sucrose in 25 mM potassium phosphate buffer (pH 7.4). Then a 20–21 g portion of minced liver was transferred to a homogenizing tube and 10 ml ice-cold 0.25 M sucrose in 25 mM potassium phosphate buffer (pH 7.4) was added and homogenized for ~3 min per portion. Using a Sigma 3K30 centrifuge with 12156-H rotor the homogenate was centrifuged at 4°C for 10 min at 1000g and then for 15 min at 12 000g and 4°C. After each centrifugation the thin floating lipid layer was removed by filtering through muslin into another centrifuge tube and the pellet was discarded. The post-mitochondrial fraction (PMF) was diluted with 7.5 times its volume of ice-cold 8 mM calcium chloride, added dropwise and constantly stirred. PMF was left to stir at 4°C for 15 min and centrifuged at 8000g for 10 min at 4°C. For each gram of original tissue, 0.15 ml of 0.25 M sucrose was added to re-suspend the pellet after removing the supernatant. The suspension called the microsome isolation was stored at ~80°C until required.

**Glucose-6-phosphatase assay**

Microsomes were diluted using sodium cholate (0.5% w/v final concentration). The assay was performed in duplicate by adding 100 µl of 160 mM sodium cacodylate as a buffer solution at pH 6.5, 15 µl of water and 25 µl of glucose-6-phosphate (final concentrations of 0, 1.56, 3.13, 4.69 and 6.25 mM) and finally 20 µl of diluted microsomes were added to initiate the assay.

The inhibitor 15 µl of VOSO$_4$ was added to give a final concentration of 6, 12, 24 and 48 µM, whereas 15 µl of Vace was added to give a final concentration of 50, 100, 150 and 200 µM. Both added instead of water. To some tubes no microsomes were added and these were the control tubes. Mixtures were vortexed and incubated at 30°C for 20 min. The reaction was stopped with the addition of 1.6 ml of Burchell’s reagent (made at a 6:2:1 ratio by volume, by adding 0.42% w/v acid molybdate, 5% w/v sodium dodecyl sulphate and 10% w/v ascobic acid, respectively). Microsomes in the control tubes (blanks) were added after adding the Burchell’s reagent.

A standard curve was constructed using 0.5 mM monopotassium dihydrogen phosphate at volumes of 0–150 µl (final phosphate concentrations 0, 20, 25, 40, 50 and 75 nM) and up to a final volume of 160 µl with 160 mM sodium cacodylate at pH 6.5. Finally, 1.6 ml of Burchell’s reagent was added and all solutions were incubated (enzyme assay and standard curve tubes) in water bath at 47°C for 2 h. Then absorbance was measured using a Cecil CE1021 spectrophotometer at 820 nm. Using direct interpolation from the standard curve the inorganic phosphate concentration was estimated.

**Tissue protein determination**

The microsome isolation was diluted with 0.5 M sodium hydroxide at a ratio of 1:100, and 1 ml of this solution was used. A blank contained 1 ml of 0.5 M NaOH. Then 5 ml of
A bovine serum albumin stock solution was prepared at a concentration of 1 mg ml\(^{-1}\) of 0.5 M NaOH and a standard curve of 0–1 mg ml\(^{-1}\) protein was prepared in 0.5 M NaOH. The standard curve solution was then processed as detailed for the tissue protein assay.
Effect of various inhibitor concentrations on enzyme activity was analysed by analysis of variance test (ANOVA).

The mean specific enzyme activity was calculated for glucose-6-phosphatase in the absence or presence of VOSO₄ and Vace, which is shown in Table 2.

### Results

The results indicate that both vanadyl compounds inhibit glucose-6-phosphatase. For VOSO₄ and no inhibitor, the two-tailed $P$ value is <0.0003 and for Vace and no inhibitor it is <0.0001.

Following the assay of microsomal fraction in a range of glucose-6-phosphate with differing VOSO₄ concentrations, the following results were obtained. Figure 6 shows the results in the form of a Lineweaver–Burk plot and so it could be ascertained whether there was any inhibition and what mechanism of inhibition there was.

The standard deviations are represented in the graph, but as the differences were so small ($\pm 0.03$), they are not very clear in the graph. This shows that in the presence of VOSO₄ there is inhibition and as the concentration of VOSO₄ increases there is greater inhibition as shown by the increasing slope of the graph.

Analysis of variance showed that the inhibition by VOSO₄ was highly significant ($P < 0.0001$).

This graph shows the $V_{\text{max}}$ value to be 1.22 $\mu$mol min⁻¹ mg⁻¹ of protein.

From this graph $K_m$ and $K_i$ values were calculated, which are shown in Table 3.

In this assay $V_{\text{max}}$ value was achieved and $K_m$ was increasing as the concentration of VOSO₄ was increased; this shows that VOSO₄ conforms to competitive inhibition Consequently, $K_i$ would be expected to decrease as increasing the concentration of VOSO₄ would mean increased affinity between the VOSO₄ and glucose-6-phosphatase.

### Table 2. Mean specific enzyme activity for glucose-6-phosphatase in the absence or presence of inhibitor indicating extent of inhibition at the maximum concentration of the inhibitor

<table>
<thead>
<tr>
<th></th>
<th>Specific enzyme activity (pmol mgprotein⁻¹ min⁻¹)</th>
<th>Inhibition (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>134.4 (±19.2, $n = 5$)</td>
<td>—</td>
</tr>
<tr>
<td>VOSO₄ (48 µM)</td>
<td>84.8 (±12.8, $n = 5$), $P &lt; 0.0003$</td>
<td>36.9</td>
</tr>
<tr>
<td>Vace (200 µM)</td>
<td>67.2 (±14.4, $n = 5$), $P &lt; 0.0001$</td>
<td>50</td>
</tr>
</tbody>
</table>

### Table 3. $K_m$ and $K_i$ values for VOSO₄

<table>
<thead>
<tr>
<th>VOSO₄ concentration (mM)</th>
<th>$K_m$ (mM)</th>
<th>$K_i$ (mM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>6.46</td>
<td>—</td>
</tr>
<tr>
<td>0.006</td>
<td>6.75</td>
<td>0.13</td>
</tr>
<tr>
<td>0.012</td>
<td>8.67</td>
<td>0.04</td>
</tr>
<tr>
<td>0.024</td>
<td>8.85</td>
<td>0.06</td>
</tr>
<tr>
<td>0.048</td>
<td>13.36</td>
<td>0.04</td>
</tr>
</tbody>
</table>

Figure 6. Lineweaver–Burk plot of the mean (±SD, $n = 5$) inverse rate of reaction of glucose-6-phosphatase against inverse concentrations of VOSO₄.
Similarly, a Lineweaver–Burk plot was constructed when Vace was added in increasing concentrations to the microsome preparations containing glucose-6-phosphate, as shown in Fig. 7.

Again the standard deviations are shown in the graph, but as the differences were small (±0.03), these are not shown clearly. This plot shows that Vace inhibits glucose-6-phosphatase. Analysis of variance showed that the inhibition by Vace was highly significant ($P < 0.0014$).

From this graph $K_m$ and $K_i$ values were calculated, which are tabulated in Table 4.

As $K_m$ varies, and $V_{max}$ is reduced, the results suggest that Vace has mixed competitive/uncompetitive inhibitory effect on glucose-6-phosphatase. As it is mixed, the inhibitor will have the affinity to glucose-6-phosphatase on its own and also the glucose-6-phosphatase and glucose-6-phosphate complex, which means two $K_i$ values can be deduced (shown in Table 5) as $K_i$ and $K'_i$.

The first set of $K_i$ results indicate the affinity between Vace and the enzyme–substrate and this value increases as the concentration of inhibitor increases, the second set of $K_i$ results show varying values as the concentration of Vace is increased. The first column suggests uncompetitive inhibition as $K_i$ values are increasing as the $K_m$ values increase.

### Discussion

Microsome isolations were deemed to be successful as the marker for microsome glucose-6-phosphatase was present. The isolations showed glucose-6-phosphatase activities of 0.42 pmol mg$^{-1}$ min$^{-1}$ of protein (SD ± 0.03, $n = 5$), slightly <0.44 pmol mg$^{-1}$ min$^{-1}$ of protein recorded in Woodward (2008), but this was sufficient to proceed with the study in confidence that microsomes were present.

Initial studies focused on determination of the kinetic parameters for bovine glucose-6-phosphatase. The results indicated a $K_m$ value of 6.46 mM and a $V_{max}$ value of 1.22 µmol min$^{-1}$ of protein. The $K_m$ value stated by Nordlie et al. (1992) is 3.1 ± 0.4 mM; however, the $K_m$ value reported
by Waddell and Burchell (1988) was much lower, but their tissue samples were obtained from rats, 0.5 ± 0.08 mM. Waddell and Burchell (1988) reported a $V_{\text{max}}$ value of 0.52 ± 0.03 µmol min$^{-1}$, whereas Nordlie et al. (1992) reported a $V_{\text{max}}$ value of 0.24 ± 0.3 µmol min$^{-1}$ mg$^{-1}$ of protein. The discrepancy between the results could be due to the differences in the source of the tissue samples. The samples reported in this study were bovine in origin and the results from the above studies were from rat liver samples.

On the contrary, Arion et al. (1972) found the $V_{\text{max}}$ value of glucose-6-phosphatase to be 1.2 µmol min$^{-1}$ mg$^{-1}$ of protein, which was similar to the $V_{\text{max}}$ value of 1.22 µmol min$^{-1}$ mg$^{-1}$ of protein reported here; however, Gonzalez-Mujica et al. (2005) reported a $V_{\text{max}}$ value of 7.59 ± 0.91 µmol h$^{-1}$ mg$^{-1}$ of protein (equivalent to 0.127 µmol min$^{-1}$ mg$^{-1}$ of protein) and a $K_m$ value of 4.78 ± 1.10 mM from glucose-6-phosphatase from intact rat hepatic microsomes. The discrepancy in these findings can be explained as their samples were intact and from rat microsomes, whereas here they were disrupted and bovine. However, the values of $K_m$ of 0.79 ± 0.27 mM and $V_{\text{max}}$ of 11.57 ± 1.55 µmol h$^{-1}$ mg$^{-1}$ of protein are equivalent to 0.193 µmol h$^{-1}$ mg$^{-1}$ of protein in disrupted microsomes. In this case the differences from the results found in this study were seemingly from the different source of microsome (they used rat and this paper looked at bovine samples).

The preparations were incubated with 48 µM of VOSO$_4$ and 200 µM of Vace separately; these concentrations were used as Kiersztan et al. (2004) had reported significant inhibition of glucose-6-phosphatase with these concentrations. In the control microsome preparation, the glucose 6-phosphatase activity was 134.4 pmol mg$^{-1}$ protein min$^{-1}$ (SD ± 19.2, n = 5), whereas in the preparation containing 48 µM of VOSO$_4$ and 200 µM of Vace, 84.8 pmol mg$^{-1}$ protein min$^{-1}$ (SD ± 12.8, n = 5) and 67.2 pmol mg$^{-1}$ protein min$^{-1}$ (SD ± 14.4, n = 5) of phosphate, respectively, was found, respectively. This shows that VOSO$_4$ at a concentration of 48 µM causes an inhibition of 36.9% on glucose-6-phosphatase and Vace at a concentration of 200 µM causes an inhibition of 50% on glucose-6-phosphatase, showing both vanadium salts act as inhibitors.

The results in Fig. 6 indicate that VOSO$_4$ is a competitive inhibitor of glucose-6-phosphatase; this can be explained by looking at what happens during competitive inhibition, the model is shown in Fig. 5a. From the Lineweaver–Burk plot, the proposed theory is that VOSO$_4$ competes against glucose-6-phosphatase for glucose-6-phosphatase. The inhibitor and the substrate both compete for the same site; therefore, when the inhibitor is present the affinity of the enzyme towards the substrate is decreased, which would be seen as an increase in the $K_m$ value as the inhibitor is added and its concentration is increased, as shown in Fig. 6. Also if the inhibition was competitive, increasing the concentration of the substrate would mean it can out-compete the inhibitor and so $V_{\text{max}}$ value could be achieved, which is why the Lineweaver–Burk plot shows that $V_{\text{max}}$ value is achieved despite adding inhibitor. The effect of a competitive inhibitor on the Lineweaver–Burk plot is both to move the x-intercept and increase the slope, and from Fig. 6 it can be seen that the slope is greater when there is VOSO$_4$ added to microsome compared with the control microsome preparation without any inhibitor and also that the different concentrations intercept the x-axis at different points. The decrease in the $K_m$ value can be explained by the fact that as the concentration of inhibitor increases, the affinity between VOSO$_4$ and glucose-6-phosphatase would increase as it is a competitive inhibitor and due to its increased availability it is more likely to out-compete the glucose-6-phosphatase for the active site on glucose-6-phosphatase.

Similarly, a Lineweaver–Burk plot was constructed when varying concentrations of Vace were added to separate microsome preparations containing glucose-6-phosphate, which is shown in Fig. 7. This plot shows that Vace inhibits glucose-6-phosphatase and increasing the concentration of Vace shows greater inhibition as shown by the increasing slopes. Subsequently, $K_m$ and $K_i$ values were deduced which are tabulated in Tables 4 and 5. These show that the $K_m$ values vary as the concentration of the inhibitor increases, and as the graph shows that $V_{\text{max}}$ value is also decreasing as the inhibitor increases in concentration, it suggests that Vace is a mixed: non-competitive and uncompetitive inhibitor of glucose-6-phosphatase. This can be understood by looking at the model of uncompetitive inhibition (shown in Fig. 5c). The $K_m$ values tend to decrease, converse to what we see in non-competitive inhibition (Fig. 5b). This can be understood using Le Chatlier’s principle, because due to the inhibitor effect, the equilibrium will shift towards forming more enzyme–substrate complex; therefore, the enzyme shows a higher affinity for the substrate even though this increased affinity does not lead to a higher $V_{\text{max}}$ value as there is no competition between substrate and inhibitor. As expected in the Lineweaver–Burk plot, uncompetitive inhibitors shift the line higher with a raised y-intercept, which is seen here as the microsome preparation without any inhibitor intercept the y-axis at 0.82 µM min$^{-1}$, whereas the preparation with 200 µM Vace intercepts the y-axis at 2.16 µM min$^{-1}$. However, the decrease in $K_m$ values is not uniform and as the $V_{\text{max}}$ value is lowered it suggest that Vace also has non-competitive inhibitor properties. The $K_i$ values in the first column of Table 5 increase, which again can be understood by Le Chatlier’s principle as an increase in uncompetitive inhibitor concentration, would shift the equilibrium to more formation of glucose-6-phosphate and glucose-6-phosphatase complex, which means reduced affinity between Vace and glucose-6-phosphatase which here is represented by increasing $K_i$ values in the first column of Table 5. The second column in Table 5 shows the affinity between Vace and glucose-6-phosphatase varies, and as the $V_{\text{max}}$ Value decreases it suggest there is also non-competitive inhibition which gives the inhibitor mixed properties.

If it were proposed the inhibition mechanism for Vace is uncompetitive you would expect the $K_i$ values to decrease, the $V_{\text{max}}$ value would not be achieved and the $K_i$ values would be expected to increase. If data from 0.1 mM were not included it
may fit this pattern, therefore it may be concluded that errors were made during the preparation of this concentration which has led to inaccurate results at that concentration, this is also shown by the zero $K_i$ values found which would suggest that Vace is not an inhibitor which we know from the other results is incorrect. Five liver samples were used in an attempt to get accurate readings, and the mean of these was used to assay the enzyme; however, more can be used to gain more accuracy.

There is no previously reported data on the mechanism of action of VOSO$_4$ or Vace on glucose-6-phosphatase; however, Li et al. (2008), looked at another vanadium compound, bis(maltolato)oxovanadium (BMOV) (IV) and its action on different phosphatase systems, protein tyrosine phosphatase 1B (PTP1B) and alkaline phosphatase. BMOV showed mixed competitive and non-competitive inhibition on PTP1B and it competitively inhibited alkaline phosphatase. It also noted that BMOV was more potent towards PTP1B than alkaline phosphatase. These data along with those found in this study suggest vanadium is a reversible inhibitor of phosphatases; however, the mechanism can vary depending on the vanadium compound and also the phosphatase system it targets.

There has been much reported data that vanadium exhibits insulin-like properties. Several clinical studies, such as Mukherjee et al. (2004), have shown that vanadium salts improve insulin action and reduce hyperglycaemia by potent inhibition of phosphatases that dephosphorylate and deactivate insulin receptor tyrosine kinase activity and provide a mechanism for enhanced insulin action. These compounds increase glucose transport in the skeletal muscle when the recognized pathway of insulin stimulated glucose transport via IRS1/2, PI 3-kinase and protein kinase B has been blocked. Accordingly, vanadium compounds have been reported to act via an alternative signalling pathway to increase the translocation or activity of glucose transporters. However, this study also suggests that this is probably not the only mechanism by which vanadium compounds enhance insulin action. A few studies, such as Kiersztan et al. (2004), support the results found in this paper, that vanadium inhibits glucose-6-phosphatase, but this paper has also described the mechanism of this action. For future studies, if these compounds can be given in quantifiable amounts to see how much would be required to control hyperglycaemia, and along with the knowledge of its mechanism of action, potential therapeutic agents can be created which attempt to regulate hyperglycaemia and treat type 2 diabetes differently than the treatments currently available which are shown in Table 1 in the Introduction section.

One of the obstacles in using vanadium for glucose management is that it is known to be harmful to humans. Glutamate pyruvate transaminase is an enzyme used to monitor liver function and if the levels of this enzyme in the plasma are raised, it indicates liver cell damage. Refat and El-Shazly (2010) have shown that the activity of this enzyme is slightly increased in a treated group with VOSO$_4$ at a dose of 100 mg (from 141.37 to 153.25 unit/dl) and slightly decreased in the group treated with vanadyl(II) sulphate at a dose of 100 mg (from 141.37 to 114.92 unit/dl). This suggests that 100 mg dose of VOSO$_4$ causes liver damage and due to the longevity of treatment required to manage diabetes, it could result in complications such as liver failure and so understandably therapeutic agents which do not carry this risk, e.g. long-term insulin, would be more preferable. However, Boden et al. (1996) showed that the administration of VOSO$_4$ at a dose of 50 mg twice daily for 4 weeks in eight patients (four men and four women) with non-insulin-dependent diabetes mellitus was well tolerated without any toxic manifestations. They also showed that VOSO$_4$ was associated with a 20% decrease in fasting glucose concentration and a decrease in the hepatic glucose output during hyperinsulinaemia. Dai et al. (1994) provided evidence for the long-term safety of VOSO$_4$ as supplementation of it at concentrations of 0.5–1.5 mg ml$^{-1}$ in water for a year did not show any significant toxic manifestations in streptozotocin-induced diabetic rats and their normal counterparts. VOSO$_4$ did not produce persistent changes in plasma aspartate aminotransferase, alanine aminotransferase and urea level, equating to no long-term liver damage, in those animals and non-specific morphological abnormalities were detected in any organs in this study. Fawcett et al. (1997) studied the effects of oral VOSO$_4$ (0.5 mg/kg per day) for a period of 12 weeks in 31 weight training athletes on their haematological parameters, including red and white cells, platelet counts, haemoglobin level, haematocrit, plasma viscosity, blood viscosity, lipids and indices of liver and kidney function. They reported that there was no effect of VOSO$_4$ treatment on haematological indices and biochemistry of the organs studied.

This paper confirms the results of Clore, Stillman and Sugerman (2000) that vanadium is an inhibitor of glucose-6-phosphatase, as well as confirming VOSO$_4$ is a competitive inhibitor of glucose-6-phosphatase, reported by Singh, Nordlie and Jorgenson (1981). Vace shows mixed competitive/non-competitive inhibition, the mechanism of action on this has not been mentioned in previous research and future studies to clarify this would be beneficial. Once the mechanism of action is confirmed further work to look at the in vivo effect of Vace would ensure the in vitro results are consistent. Overall, the results reported here uphold the hypothesis and also support what has been discovered previously. From the results obtained in this study together with the evidence published regarding the benefit of vanadium compounds in lowering plasma triglycerides, vanadium compounds are potentially important therapeutic agents. Further research into the mode of action of these compounds and evidence of toxicity would be an important step to make in developing treatments for type 2 diabetes.

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The author has completed a Biochemistry and Microbiology degree in June 2011, during which this research article was submitted. The author is currently working towards a PGCE in chemistry and aims to become a secondary school teacher. Her interest consists of reading fiction, cooking and taking care of her family.

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