

**EXPERT REVIEW OF GLUCOSAMINE AND
GLUCOSE TOLERANCE IN NORMAL, PRE-DIABETIC
AND DIABETIC INDIVIDUALS**

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1.0 OBJECTIVE

At the request of Cargill Incorporated, an Expert Panel (the "Panel") of independent scientists, qualified by their relevant national and international experience and scientific training, was convened on April 10, 2007 to conduct a critical and comprehensive evaluation of the available pertinent data and information, and determine whether glucosamine hydrochloride, under the conditions of intended use as a "novel" food ingredient in pasteurised fruit juices and fruit juice products (including tomato and tomato mixtures and fruit "smoothies"); dehydrated instant drink mixes; fermented milk-based products, yoghurts and fromage frais; sports drinks and iced tea drinks, would be expected to affect glucose tolerance/insulin sensitivity in normal, pre-diabetic, and diabetic individuals.

The Panel consisted of the below-signed qualified scientific experts: Dr. James W. Anderson, MD (University of Kentucky, Lexington); Dr. Anthony R Leeds, MB BS MSc CBiol FIBiol RNutr (King's College, London); Prof. Vincent Marks, DM, FRCP, FRCPath (University of Surrey). *Curricula vitae* evidencing the Panel members' qualifications for evaluating the safety of food ingredients, and expertise in glucose metabolism are provided in Appendix B.

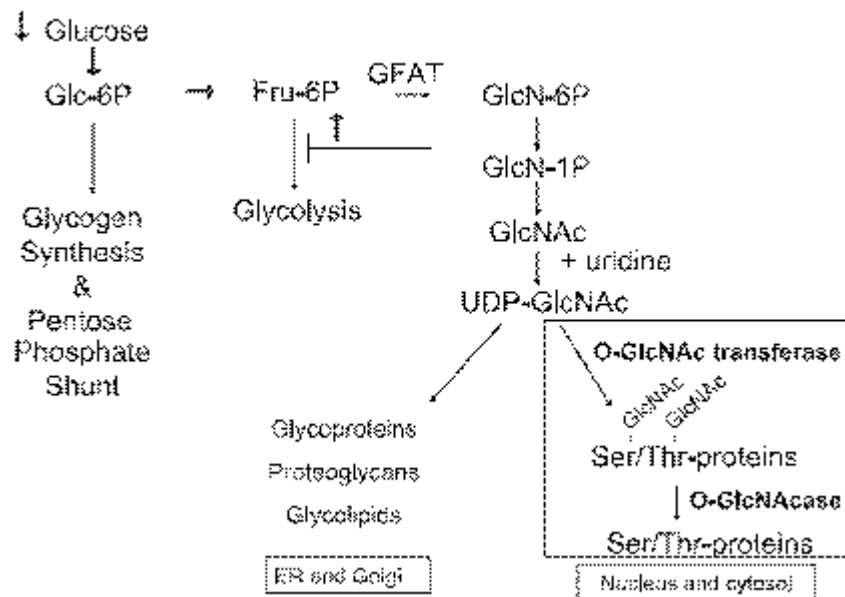
The Panel, independently and collectively, critically examined a comprehensive package of publicly available scientific information and data on glucosamine, compiled from the literature and other published sources through April 2007. The data evaluated by the Panel was collected using the electronic search tool, DIALOG, consisting of several databases, including MEDLINE®, TOXFILE, AGRICOLA, JICST-Eplus, BIOSIS Previews®, and EMBASE®. To identify all available literature relevant to the safety assessment of glucosamine in normal, pre-diabetic and diabetic individuals, the following terms were used in the search criteria: glucosamine and diabetes; glucosamine and glucose metabolism; glucosamine and glucose tolerance; glucosamine and glucose intolerance; glucosamine and impaired glucose tolerance; glucosamine and insulin resistance; glucosamine and insulin sensitivity; glucosamine and oral glucose tolerance test; glucosamine and hyperglycaemia; glucosamine and high blood sugar. The results of these searches in relation to the above objective are reviewed below with a particular emphasis on the available human clinical studies.

2.0 PROPOSED MECHANISM(S) AND ANIMAL AND *IN VITRO* STUDIES

The possibility that glucosamine may affect glucose homeostasis in humans has been proposed based on observations by a number of researchers who reported that glucosamine could alter glucose homeostasis and induce insulin resistance in rats when glucosamine is infused intravenously at high doses (Baron *et al.*, 1995; Rossetti *et al.*, 1995; Hawkins *et al.*, 1997; Virkamäki *et al.*, 1997; Holmäng *et al.*, 1999; Kim *et al.*, 1999; Patti *et al.*, 1999; Spampinato *et al.*, 2003). Adams (1999) reviewed, in an editorial, the use of glucosamine for treating arthritis. He suggested, on the basis of experimental results in rats, that it might be diabetogenic by inhibiting insulin secretion. He used an unrealistic chemical analogy with the diabetogenic antibiotic, streptozotocin, to justify this assumption. Adams failed to mention that the adverse effects of glucosamine in laboratory animals occurred only at blood glucosamine levels 100- to 1,000-fold higher than can be achieved in man by oral administration. For example, the dose used to induce insulin resistance and glucose intolerance in laboratory animals is usually 30 µmol/l per kg body weight per minute, which equated to plasma concentrations of 800 µmol/l (Patti *et al.*, 1999). The bioavailability of oral glucosamine is low, and less than 20% of an oral dose is absorbed in all species for which bioavailability determinations have been made (Adebowale *et al.*, 2002; Aghazadeh-Habashi *et al.*, 2002a,b; Du *et al.*, 2004; Lavery *et al.*, 2005). Since maximum plasma concentrations of glucosamine following a 1,500 mg oral dose are in the region of 8 µmol/l (Roda *et al.*, 2006), the glucosamine concentrations used in the animal studies is roughly 100 times the maximum estimated plasma concentration in humans using glucosamine as a dietary supplement. Evidence that glucosamine can affect glucose metabolism at lower intravenous infusion doses (3 µmol/l per kg body weight per minute) has been reported, although the plasma glucosamine levels were still roughly 20-fold in excess of those expected in humans (Hawkins *et al.*, 1997). In addition, the majority of studies above reported glucosamine induced effects primarily under euglycaemic conditions and there is some evidence that the effect does not occur in hyperglycaemic diabetic animals (Rossetti *et al.*, 1995). Glucosamine has also been shown to affect glucose and insulin homeostasis in sheep using high intravenous glucosamine doses (Robertson *et al.*, 2005). In contrast to the abundance of literature reporting that intravenous infusion of glucosamine can impair glucose and insulin homeostasis in animals, this observation seems limited to intravenous glucosamine exposures, as several animal studies conducted in, rabbits and dogs using oral glucosamine doses ranging from 50 to 2,149 mg/kg body weight (2- to 90-fold higher than usual doses for humans) failed to alter fasting glucose levels (Stender and Astrup, 1977; Setnikar *et al.*, 1991; McNamara *et al.*, 1996). Moreover, Echard *et al.* (2001) investigated the effects of oral glucosamine in rats highly sensitive to sugar-induced insulin resistance (the spontaneously hypertensive rat) at doses of 3 to 7 times the corresponding human intakes from supplemental use, and also found that orally administered glucosamine did not alter glucose tolerance or insulin sensitivity.

Mechanistically it has been proposed that the effect observed in animals is mediated by glucosamine interfering with hexosamine biosynthesis in the cell (Figure 2-1). In healthy animals under normal conditions, glucose entering cells is phosphorylated by glucokinase to glucose-6-phosphate (Glc-6P). Depending on the energy requirements of the cell, Glc-6P phosphate is then used as a substrate for glycogen synthesis, as a substrate for NADPH synthesis following metabolism via the pentose phosphate shunt, or alternatively, Glc-6P is metabolized to fructose-6 phosphate (Fruc-6P) and proceeds through the glycolysis pathway. The hexoseamine pathway is a minor branch of the glycolysis pathway and comprises about 3% of the total glucose entering it (Marshall *et al.*, 1991). This pathway is regulated by the first and rate limiting enzyme glutamine:fructose-6-phosphate (GFAT), which catalyzes the conversion of Fruc-6P to glucosamine-6 phosphate (GlcN-6P). GlcN-6P is then metabolized to UDP-N-acetylglucosamine (UDP-GlcNAc). Since UDP-GlcNAc has been shown to both directly and indirectly mediate glucose metabolism and insulin action, the hexosamine pathway may function as a nutrient sensor regulating glucose utilization in the cell (Buse, 2006). For example, UDP-GlcNAc is an inhibitor of GFAT and a substrate of O-GlcNAc transferase (OGT), which mediates the glycosylation of various proteins involved in regulating glucose uptake and insulin activity (Buse, 2006). It is believed that exogenous glucosamine, at high levels, can bypass GFAT in the cell and cause a metabolic flux through the hexosamine pathway in a manner that is not reflective of current glucose homeostasis (Marshall *et al.*, 1991). The result of continuous glucosamine flux through the hexosamine pathway is the production of increased amounts UDP-N-acetylglucosamine, and process that would eventually result in the down regulation of glucose uptake and insulin insensitivity (Buse, 2006; Stumpf and Lin, 2006). A number of *in vitro* and *in vivo* experiments have proposed more specific mechanisms whereby glucosamine induced increases in hexosamine metabolism affect glucose and insulin homeostasis. For example, it has been reported that glucosamine can induce insulin resistance by affecting glucose receptor (GLUT-4) translocation in skeletal muscle, an effect that may be due to impaired GLUT-4 phosphorylation (Baron *et al.*, 1995; Spampinato *et al.*, 2003). Other authors have reported that glucosamine can impair glycogen metabolism, or induce insulin resistance by altering the activity of various insulin mediated signal transduction molecules (Kim *et al.*, 1999; Patti *et al.*, 1999). The inhibition of glucokinase is also another important, and perhaps under appreciated potential mechanism to explain the observed effects in animals, and under *in vitro* conditions, the inhibition of glucokinase activity by glucosamine, could alter glucose uptake by decreasing glucose phosphorylation, which in turn would impair glucose cycling, evidence supporting this effect has been presented in the literature (Balkan and Dunning., 1994; Monauni *et al.*, 2000). Similar to animal models, *in vitro* effects on glucose homeostasis induced by glucosamine generally require concentrations in the range of 500 to 20,000 $\mu\text{mol/l}$ and most studies use glucosamine at concentrations of at least 2,000 to 10,000 $\mu\text{mol/l}$ to observe significant effects (Balkan and Dunning., 1994; Ciaraldi *et al.*, 1999; Nelson *et al.*, 2000; Sakai and Clemmons, 2003; Marshall *et al.*, 2005). This concentration range is roughly 250 to 1,250 times the expected plasma concentrations anticipated with oral glucosamine administration in humans.

Overall, it is clear that the intravenous administration of glucosamine adversely alters glucose tolerance and insulin sensitivity in animals, and numerous *in vitro* models in human and animal tissues have been proposed to explain the observed effects. However, the requirement for high glucosamine concentrations to elicit effects makes extrapolation of these experiments to humans difficult. Mechanistically, observations that glucosamine can induce glucose intolerance and insulin insensitivity may not be relevant to humans due to the significant species differences in glucokinase affinity for glucose and glucosamine. In the rat, the affinity of glucokinase for glucose and glucosamine are roughly equal, with a slight increase in affinity favouring that of glucose over glucosamine. The glucokinase affinity constant (K_m) for glucosamine is reported to be ~8 mM (Oguchi *et al.*, 1975; Oguchi *et al.*, 1977) vs. values in the range of 10 to 20 mmol/l for glucose (Parry and Walker, 1967; Grossman *et al.*, 1974). Storer and Cornish-Bowden (1976) have argued that the glucokinase-glucose K_m values reported above (10 to 20 mM) are inaccurate, and that the true K_m value of rodent glucokinase for glucose is 5 mM, a value that infers that the affinity of the enzyme for glucose relative to glucosamine are similar (Storer and Cornish-Bowden, 1976). This observation is supported by Oguchi *et al.* (1977) who show that the phosphorylation of glucosamine by rat liver glucokinase is not affected by physiological concentrations of glucose (5 mM). In contrast, the affinity of glucokinase for glucosamine and glucose differ by an order of magnitude in humans, such that the affinity of glucokinase for glucose is 10-fold higher than that of glucosamine (Xu *et al.*, 1995). The affinity of glucokinase for glucose is such that the enzyme is maximally operative at normal physiological plasma glucose concentrations ensuring that glucose phosphorylation maintains a gradient for glucose transport (Mueckler, 1993). Thus, given the apparent species differences in the affinity of glucokinase for glucose and glucosamine it is not surprising that experimental evidence shows that rodents are responsive to glucosamine's effects on glucose and insulin sensitivity, whereas humans are not. Mechanistically, the species differences in enzyme affinity suggests that it is not appropriate to extrapolate findings in animal models pertaining to glucosamine metabolism and insulin sensitivity to potential effects in humans consuming glucosamine.



Adapted from Buse, 2006.

Figure 2-1 Schematic Representation of Glucose Metabolism and the Hexosamine Pathway

3.0 HUMAN STUDIES

Twenty studies were identified from the literature search in which the effects of glucosamine on various measures of glucose and insulin homeostasis were reported (Appendix A). Of these studies, 10 were directly designed to investigate the effects of glucosamine on glucose metabolism or insulin resistance. In total, only 2 peer-reviewed studies suggest that glucosamine impairs glucose tolerance and reduces insulin sensitivity. Detailed reviews of the relevant studies are summarized below, and the reader is directed to the table in Appendix A, for further information that may not be included in the summarized text below.

Evidence that glucosamine may affect glucose metabolism in healthy individuals under similar conditions used in the animal studies is presented by Monauni *et al.* (2000). The investigators conducted a study in 10 healthy volunteers by sequentially performing an intravenous glucose (plus [2-³H] glucose) tolerance test (IVGTT) and a euglycaemic insulin clamp during a saline infusion, and low, or high glucosamine infusions (1.6 and 5 µmol/kg minute respectively). Catheters were inserted into a vein in the wrist, and saline or low or high glucosamine was infused at the above rates over a time course of 360 minutes (-60 to 300 minutes). The resulting plasma glucosamine concentrations in the low infusion glucosamine group increased steadily to a maximum of 570±140 µmol/l in the low infusion glucosamine group, and to a maximum of 1,150±180 µmol/l in the high glucosamine group.

Starting at t=0, the IVGTT was performed, and once plasma glucose levels returned to baseline, the insulin clamp procedure was initiated.

Following the IVGTT, glucosamine at neither dose displayed any effect on serum insulin levels, glucose stimulated insulin secretion, or readily reversible insulin intolerance; however, both plasma glucose and tritiated plasma glucose concentrations following high-dose glucosamine were slightly higher ($P < 0.01$) suggesting that glucose tolerance was slightly impaired. Mechanistically the authors suggested that the effect was consistent with glucosamine acting as a competitive inhibitor of liver glucokinase, which would result in a reduced rate of glucose phosphorylation, and glucose cycling. In contrast to the effects observed following IVGTT, glucosamine did not affect any parameter of glucose metabolism, or glucose storage during the euglycaemic insulin clamp.

It is clear from the study above that high intravenously derived plasma levels of glucosamine can modestly affect glucose metabolism in humans; however, the effect required plasma glucosamine levels that were ~100 times the levels expected following oral glucosamine supplementation. Thus, it seems reasonable to conclude that the consumption of glucosamine at even high supplemental doses (3,000 mg per day) is unlikely to induce a similar response as observed in this study. Although some effects of high glucosamine on glucose metabolism parallel observations reported in animal studies, unlike rats, high intravenous glucosamine doses did not impair glucose or insulin metabolism under euglycaemic conditions. The authors were unclear why this discrepancy existed, and indicated that it was not likely due to glucosamine doses that were too low, since the plasma levels reached in this study (1,150 $\mu\text{mol/l}$) were greater than those reported in animal studies (800 $\mu\text{mol/l}$).

In another intravenous study, Pouwels *et al.* (2001) investigated the effects of intravenous glucosamine on glucose tolerance or insulin sensitivity in 10 healthy male normoglycaemic volunteers using euglycaemic hyperinsulinaemic clamp analysis methods (Pouwels *et al.*, 2001). No effect on glucose or insulin tolerance was observed. The dose of glucosamine used in this study resulted in a plasma glucosamine concentration of 150 $\mu\text{mol/l}$ and inability of glucosamine to affect glucose metabolism is therefore consistent with the above study by Monauni *et al.* (2000) where plasma glucosamine levels of 570 $\mu\text{mol/l}$ also failed to alter glucose homeostasis.

Impaired glucose tolerance following oral consumption of glucosamine has been recently suggested in the study by Biggee *et al.* (2007). This study was designed to investigate the effect of oral glucosamine sulphate on serum glucose and insulin during an oral glucose tolerance test, and was conducted in 16 osteoarthritis patients. Following an overnight fast, the subjects were cannulated, and blood samples were taken every 15 to 30 minutes during 3-hour period following the ingestion of glucose (75 g) with or without 1,500 mg of glucosamine sulphate. During the experiment it was discovered that 3 subjects were undiagnosed diabetics, and sub-group analysis of the data was then performed using the 13 normoglycaemics and 3 undiagnosed diabetics as separate groups. Oral glucosamine did

not affect glucose or insulin area under the curve (AUC) values in the 13 normoglycaemic subjects following the oral glucose tolerance test. Serum AUC values were 29 ± 27 vs. 39 ± 22 mg minute/ml for the glucosamine + glucose vs. glucose only treatment. However in the three subjects identified as undiagnosed diabetics, oral glucosamine consumption during the glucose challenge resulted in an increase in glucose AUC values by 32% ($P < 0.05$) relative to the glucose only treatment. Serum AUC values were 191 ± 94 vs. 145 ± 86 mg min/ml for the glucosamine + glucose vs. glucose only treatment. No change in insulin AUC values for glucose challenge with or without glucosamine administration was observed. The authors concluded that oral glucosamine may decrease glucose tolerance in subjects with undiagnosed diabetes. However, a critical analysis of the study reveals several obvious limitations that make such inferences difficult to substantiate. First, is the small sample size and large inter-individual variations in the endpoints (glucose and insulin AUC values) observed for both normoglycaemics and in those with undiagnosed diabetes, which indicates that the statistical quality of the results is poor. In addition, the high intra-person variability in the results of the oral glucose-tolerance test that were observed in normoglycaemic subjects and those classified as undiagnosed diabetics is common following a single oral glucose challenge, and It is well established that substantial bias can be introduced into studies where subjects are classified into a particular glucose tolerance category basis on the results of a single oral glucose-tolerance test (Meigs *et al.*, 1998).

Secondly, and perhaps the most important caveat to the study, is the allocation of the glucosamine “responders” as a separate undiagnosed diabetic subgroup for endpoint analysis. The bias associated with the failure to analyze data on an intent-to-treat basis is well acknowledged. Moreover a careful examination of the 2-hour glucose levels reveals that subjects were included in the subgroup as undiagnosed diabetics based on the results of a desired outcome, *i.e.*, an elevated 2-hour glucose level following glucosamine + glucose treatment. Had inclusion of subjects into the subgroup been based on the results of the glucose tolerance test in the absence of glucosamine (which seems more reasonable), there would be no way to rationalize inclusion of subject No. 5 in the group. It appears as if the authors have tried to hide this by reporting the data in graphical form only, and by failing to include error bars in the control graphs. The authors state that subjects were categorized as suffering from diabetes based on World Health Organization (WHO) criteria. Based on WHO definitions subjects with 2-hour glucose values of >200 mg/dl are categorized as diabetic and those with values >140 and <200 mg/dl are considered to display impaired glucose tolerance. Based on the graphical data subject No. 5 had a 2-hour glucose level below 140 mg/dl in the absence of glucosamine, and therefore should have been included in the control rather than the undiagnosed diabetic group. In addition, if subject No. 5, was in fact glucose intolerant, he/she would be expected to display much higher T=0 insulin level relative to the controls, and further questions the appropriateness of including subject No. 5 into the diabetic/glucose intolerant group. Had subject No. 5 been included in the control group, a glucosamine effect could not have been reported, and the study would likely not have been published. The bias in analyzing data in this manner is substantial, and cannot be ignored.

The authors also provide an explanation for the failure of many studies to show glucosamine effects on glucose tolerance by suggesting that their study was unique in that the glucose tolerance test was performed within 5 minutes following glucosamine consumption rather than in the morning following an overnight fast when plasma glucosamine levels would be low. Furthermore, the authors also state that this discrepancy is important in that immediately following the consumption of 1,500 mg of glucosamine, 1,250 mg (based on the assumption that 90% of orally administered glucosamine is absorbed) of glucosamine would travel directly through the portal system to provide the extracellular 300 to 400 ml of liver water with a glucosamine concentration as high as 5 to 20 mmol/l. The authors further infer that the apparent 1,000-fold difference in glucosamine concentration than levels reached in the peripheral circulation following oral glucosamine dosing is due to first pass metabolism by the liver. Biggee *et al.* (2007) concluded that intravenous studies fail to show significant glucosamine effects because intravenous glucosamine administration by-passes the liver and therefore does not result in liver exposure to as high glucosamine concentrations as those that occur immediately following oral administration. This assumption is based entirely on the belief that 90% of orally administered glucosamine is absorbed and that the low bioavailability of glucosamine in animals (2.5 to 20%) is due to first-pass metabolism by the liver; an argument that is not supported by experimental data. The bioavailability of glucosamine has been reported to be 10% in dogs (Adebowale *et al.*, 2002), 2.5 to 6% in horses (Du *et al.*, 2004; Laverty *et al.*, 2005), and 20% in rats (Aghazadeh-Habashi *et al.*, 2002a). In a study in humans, incorrectly described as measuring glucosamine bioavailability, it was the ¹⁴C label rather than the intact glucosamine molecule that was measured, (Setnikar *et al.*, 1993). Glucosamine is a charged molecule that is unlikely to be efficiently absorbed, and studies conducted by Aghazadeh-Habashi *et al.* (2002a,b) in the rat using orally administered glucosamine and butyl-glucosamine show that the poor bioavailability is due to extensive gastrointestinal metabolism and not due to first-pass metabolism by the liver. Biggee *et al.*'s belief that 90% of orally administered glucosamine is readily absorbed is based on the work of Setnikar *et al.* (2001), in which ¹⁴C labelled glucosamine was administered by mouth and 90% of the label was excreted as CO₂ in breath and in urine over a 120-hour period. No qualitative measurements of intact glucosamine were made during this study, and in light of the work of Aghazadeh-Habashi *et al.* (2002a,b), it is reasonably certain that the apparent almost complete absorption of glucosamine (over a 120-hour period) was predominantly attributed to the absorption of bacterial metabolites of glucosamine from the large intestine. A number of *Bacteroides* sp. found in the colon are known to ferment D-glucosamine (Salyers *et al.*, 1977). The poor absorption of glucosamine is also highlighted by 2 studies where large oral doses of glucosamine were administered: Persiani *et al.* (2005) show that when an oral dose of glucosamine is doubled from 1,500 to 3,000 mg no significant increase in plasma concentrations of glucosamine was observed; and in 6 healthy volunteers consuming in excess of 5 times (7,540 mg) typical supplemental doses, plasma levels of glucosamine analyzed over a 180-minute period did not increase above the detection limit of the analysis assay (0.0167 μmol/l) (Setnikar *et al.*, 2001).

Two preliminary reports published only as abstracts were identified in the literature where oral glucosamine was reported to adversely affect glucose metabolism (Almada *et al.*, 2000; Pham and Scofield, 2005). As part of a secondary analysis of a study investigating the effect of glucosamine on back pain, Almada *et al.* (2000) reported the effects of glucosamine on glucose metabolism following the administered glucosamine (1,500 mg/day) or placebo to 15 subjects (6 glucosamine, 9 placebo) for a period of 12 weeks. No differences in fasting glucose or insulin levels were reported at week 12 between groups. However a significant ($P<0.01$) between group increase in fasting insulin levels at week 12 relative to baseline was reported. Pham and Scofield (2005) conducted a study in 32 subjects to determine whether insulin resistance occurs in non-diabetics after 6 weeks of glucosamine administration (1,500 mg/day). At week 6 the authors reported that log HOMA values were increased by 25% ($P<0.008$) following glucosamine treatment, and that QUICKI values were decreased by 5% ($P<0.017$). The study by Pham and Scofield (2005) did not contain a control group, and in the study by Almada *et al.* (2000), although a significant change in glucose and insulin levels were observed for the glucosamine group at week 12 relative to baseline, no statistically significant differences relative to controls was observed. More importantly, both of the studies reviewed above were reported as non-peer reviewed articles published in abstract form only, and over a 6-year time span peer-reviewed publications of the data have not appeared in the literature. The significance of the results should therefore be interpreted with caution.

The best evidence presented to date that glucosamine does not affect glucose tolerance or insulin sensitivity is presented by Muniyappa *et al.* (2006). These authors conducted a well designed, randomized, double-blind, placebo controlled crossover study to assess the effect of 6 weeks of oral glucosamine (1,500 mg/day) on insulin resistance and endothelial dysfunction in 20 lean and 20 obese healthy subjects. Insulin resistance was determined using highly sensitive euglycaemic clamp methodology, and endothelial function was determined by measuring brachial artery flow and forearm skeletal muscle microvascular recruitment. At baseline, it was observed that the obese subjects displayed significant insulin resistance ($P<0.0001$) relative to lean subjects as well as significantly increased endothelial cell dysfunction ($P<0.04$). Therefore this study is an excellent means to determine the effect of glucosamine in both subjects with apparent insulin resistance and in non-insulin resistant subjects under well controlled conditions. Based on a lack of significant differences between groups for the various analytical endpoints, the authors concluded that glucosamine does not significantly worsen insulin resistance or endothelial dysfunction in lean and obese subjects.

Similar observations to those reported by Muniyappa *et al.* (2006) were also observed by Yu *et al.* (2003) who investigated the effects of 4 weeks of oral glucosamine sulphate (1,500 mg/day) on insulin sensitivity and glucose response in 7 lean and 7 obese subjects. The obese subjects displayed impaired baseline insulin resistance, and 1 lean subject and 3 obese subjects displayed impaired glucose tolerance at baseline. Following 4 weeks of glucosamine administration no differences in fasting glucose or insulin levels were observed between lean and obese subjects, and the pooled (lean + obese) results from the glucose

challenge and insulin sensitivity analyses did not differ between baseline and week 4. Sub-group analysis of the data based on BMI or glucose tolerance also failed to show a glucosamine effect. In an early study by Weiden and Wood (1958) a similar lack of glucosamine effect was reported for a subgroup of 6 poorly controlled hyperglycaemic diabetic subjects, where no differences in blood glucose levels were observed following an oral glucose tolerance test relative to normoglycaemics after intravenous glucosamine infusion.

In a double blind placebo controlled study by Tannis *et al.* (2004) conducted in 19 healthy male and female subjects, daily glucosamine (1,500 mg) for 12 weeks had no effect on fasting glucose or insulin levels and no change in glucose tolerance was observed following glucose challenge. The effects of acute high-dose oral glucosamine were investigated by Lafèrrere *et al.* (2004) in 20 healthy non-obese subjects with normal glucose tolerance. Six subjects received 3,000 mg of glucosamine and 5 subjects received 6,000 mg of glucosamine in the morning following an overnight fast; 9 control subjects were studied under the same conditions. The authors observed that acute high-dose glucosamine did not modify glucose or insulin levels.

Controlled studies investigating the effects of glucosamine are limited in that effects on glucose and insulin were measured as part of the safety assessment and not as the primary outcomes; however one study was identified in which the effects of glucosamine on glucose and insulin homeostasis in diabetic subjects were directly monitored (Scroggie *et al.*, 2003). The investigators administered glucosamine (1,500 mg/day) and chondroitin sulphate (1,200 mg/day) to 26 male and female type II diabetics for 90 days (12 subjects received placebo treatment). Four subjects from the treatment group dropped out of the study; however, the authors determined that reasons were not due to glucosamine or a worsening of glycaemic control. Haemoglobin A_{1c} (HbA_{1c}), a measurement related to mean blood glucose levels during the preceding 3 months, was analyzed on day 90 and no significant difference was found between subjects using glucosamine relative to controls. In a study by Tapadinhas *et al.* (1982) where 516 males and 692 females received glucosamine (1,500 mg/day) for 6 to 8 weeks, 92 diabetics, and 74 patients receiving hypoglycaemic medication were included in the study, and the authors reported no variation in tolerability in the presence of diabetes or with the treatment of hypoglycaemic medication.

Two studies investigated the long-term effects of glucosamine (1,500 mg once daily) over a 3-year period in subjects (>50 years of age) with osteoarthritis (Reginster *et al.*, 2001; Pavelká *et al.*, 2002), and both studies were randomized placebo controlled studies using ~200 subjects in each trial. In the study by Pavelká *et al.* (2002) 4 patients developed diabetes during the study, with 3 subjects in the placebo group developing diabetes relative to one subject in the glucosamine group. In the study by Reginster *et al.* (2001) the drop-out rates were equal in both groups, and no significant difference in reasons for drop-out was reported. In addition, routine laboratory monitoring did not show any significant changes in glycaemic homeostasis, with fasting plasma glucose levels decreasing for the glucosamine group relative to the placebo.

The recent Glucosamine/Chondroitin Arthritis Intervention Trial (GAIT) commissioned by the National Institutes of Health (USA) to assess the safety of glucosamine was conducted in a large number of subjects with osteoarthritis (average age 59 years) using a randomized double-blind placebo controlled study design over a 6-month period (Clegg *et al.*, 2006). Two hundred forty-two (242) subjects were randomized to receive glucosamine treatment (1,500 mg/day), and 313 subjects were randomized to the placebo group. The study included diabetic subjects (number not reported) and patients with diabetes had fasting plasma glucose or glycosylated haemoglobin levels monitored during the study; no significant glucosamine induced changes in these parameters were reported. In addition, no increased risk of cardiovascular disease was observed in diabetics receiving glucosamine (an effect that would be a result of impaired blood glucose management).

Finally, a number of additional studies have been conducted where full glucose measurements and/or clinical chemistry monitoring was conducted, and no significant changes as a result of glucosamine administration were reported (Crolle and D'Este, 1980; Drovanti *et al.*, 1980; Pujalte *et al.*, 1980; D'Ambrosio *et al.*, 1981; Rovati, 1992; Noack *et al.*, 1994; Giordano *et al.*, 1996; Qiu *et al.*, 1998; Hughes and Carr, 2002).

4.0 SUMMARY

Intravenous glucosamine administration impairs glucose tolerance and reduces insulin sensitivity under euglycaemic conditions in rodents. The effect observed in rodents occurs at plasma concentrations that are approximately 100- to 1,000-fold in excess of the maximum plasma levels expected following oral glucosamine supplementation in humans. Similarly, glucosamine induced effects on glucose metabolism and insulin sensitivity *in vitro* also require high glucosamine concentrations; concentrations that are in the range of 250 to 1,250 times typical plasma levels in humans consuming glucosamine. Nevertheless, though rodents are sensitive to high plasma levels of glucosamine achievable only by intravenous infusions, oral glucosamine administration to animals has not been reported to alter glucose metabolism or insulin sensitivity at doses that are 2- to 90-fold greater than the usual oral doses in humans. In addition, significant species differences exist for the affinity of glucokinase for glucose and glucosamine suggesting that observations observed in rodents are not relevant to humans. Thus, given the apparent discrepancy in the sensitivity of rodents to glucosamine under *in vivo* conditions, and the fact that oral glucosamine does not alter glucose metabolism in animals responsive to glucosamine administered intravenously, it seems highly unlikely that the consumption of glucosamine at proposed intakes not exceeding 1,500 mg per day would affect glucose tolerance or insulin sensitivity in healthy individuals.

Clinical studies are in accordance with the above conclusion. Evidence presented by Monauni *et al.* (2000) show that the plasma glucosamine concentration threshold for glucosamine adversely effecting glucose metabolism is very high. Intravenous glucosamine infusion resulting in a glucosamine concentration of 570 µmol/l has no adverse effects on glucose tolerance or insulin sensitivity in normoglycaemic healthy individuals. Following oral

consumption of glucosamine at standard supplemental levels, maximum plasma levels are ~8 µmol/l, well below the level where modest impairment in glucose tolerance was observed following intravenous glucosamine administration (1,150 µmol/l). Persiani *et al.* (2005) show that the consumption of glucosamine at doses twice the typical supplemental use (3,000 mg) did not significantly increase plasma AUC glucosamine levels above those achieved using the standard 1,500 mg dose. Thus, the possibility of increased glucosamine exposure above the 90th percentile expected intakes, occurring from individuals who may consume glucosamine under the proposed uses in conjunction with supplemental use, would not result in a significant increase in internal glucosamine exposures.

A recent study by Biggee *et al.* (2007) has raised the possibility that glucosamine may affect glucose tolerance and insulin sensitivity in un-diagnosed diabetics. However, the small sample size, large inter-individual variation in glucose and insulin levels, and obvious bias associated with both the use of sub-group analysis and method of subject inclusion into the sub-groups do not substantiate the inferences made by the authors. In addition, the authors also make several incorrect assumptions about the absorption efficiency of glucosamine in humans. More importantly, two studies have been conducted that included subjects with poor glucose tolerance using highly sensitive euglycaemic insulin clamp analysis methodology (Muniyappa *et al.*, 2006); no worsening of glucose tolerance or insulin sensitivity was observed (Yu *et al.*, 2003; Muniyappa *et al.*, 2006). Given the inherent bias in the Biggee *et al.* (2007) study, the results obtained by Muniyappa *et al.* (2006) and Yu *et al.* (2003) are more scientifically sound than those of Biggee *et al.* (2007), and suggest that the consumption of glucosamine as a Novel Food ingredient, will not affect glucose tolerance or insulin sensitivity in subjects with undiagnosed diabetes. Also given the significantly higher affinity of human glucokinase for glucose over glucosamine, there is no mechanistic rationale to infer that diabetics would be more sensitive to glucosamine relative to normoglycaemic individuals.

Overall, 16 chronic studies have evaluated fasting blood glucose values of humans treated with glucosamine. Fasting glucose values decreased non-significantly from 92.9 to 89.9 for values reported for 5 trials (Anderson *et al.*, 2005). Long-term glucosamine use (1,500 mg for 3 years) in over 200 subjects did not result in an increased incidence of diabetes, and in fact decreased incidences of diabetes, and decreases in fasting glucose values relative to baseline were reported at the end of the trials (Reginster *et al.*, 2001; Pavelká *et al.*, 2002). The recent GAIT was commissioned by the National Institutes of Health (USA) to assess the safety of glucosamine (Clegg *et al.*, 2006). The GAIT enrolled 317 subjects with osteoarthritis (average age 59 years) into the glucosamine arm and 242 completed the 6-month trial. The study included diabetic subjects; fasting plasma glucose measurements were made in all subjects. No significant changes in blood glucose values were reported (Clegg *et al.*, 2006). In aggregate reports from 14 trials, including 1,299 subjects treated for an average of 24 weeks (598 patient years of observation) indicated that there were no significant changes in blood glucose values (Anderson *et al.*, 2005; Clegg *et al.*, 2006). For the entire group of 34 studies of chronic glucosamine administration, including predominantly

older subjects, three subjects developed diabetes with placebo treatment and two subjects developed diabetes with glucosamine treatment.

5.0 CONCLUSIONS

Glucosamine is a widely used dietary supplement. Glucosamine is synthesized in humans and is effectively metabolized (Anderson et al., 2005; Muniyappa et al., 2006). In clinical trials it has been administered to many subjects with type 2 diabetes but the numbers have not been quantified by reporting investigators. Fasting blood glucose values have decreased slightly for subjects – including diabetic and non-diabetic individuals – treated for periods up to three years. No data are available to document that oral administration of glucosamine, at any dose level, has adverse effects on blood glucose levels, glucose metabolism or insulin sensitivity.

We, the Expert Panel, have independently and collectively, critically evaluated the data and information summarized above and conclude that based on the weight of scientific evidence, there seems to be no reason to restrict the use of glucosamine for individuals at risk for diabetes or for diabetic individuals.

6.0 REFERENCES

- Adams, M.E. 1999. Hype about glucosamine. *Lancet* 354(9176):353-354.
- Adebowale, A.; Du, J.; Liang, Z.; Leslie, J.L.; Eddington, N.D. 2002. The bioavailability and pharmacokinetics of glucosamine hydrochloride and low molecular weight chondroitin sulfate after single and multiple doses to beagle dogs. *Biopharm Drug Dispos* 23(6):217-225.
- Aghazadeh-Habashi, A.; Sattari, S.; Pasutto, S.S.; Jamali, F. 2002a. Single dose pharmacokinetics and bioavailability of glucosamine in the rat. *J Pharm Pharmaceut Sci* 5(2):181-184.
- Aghazadeh-Habashi, A.; Ibrahim, A.; Carrman, J.; Anastassiades, T.; Jamali, F. 2002b. Single dose pharmacokinetics and bioavailability of butyryl glucosamine in the rat. *J Pharm Pharmaceut Sci* 9(3):359-364.
- Almada, A.L.; Harvey, P.; Platt, K. 2000. Effect of chronic oral glucosamine sulfate upon fasting insulin resistance index (FIRI) in non-diabetic individuals. *FASEB J* 14(4):A750 [Abstract No. 521.15].
- Anderson, J.W.; Nicolosi, R.J.; Borzelleca, J.F. 2005. Glucosamine effects in humans: a review of effects on glucose metabolism, side effects, safety considerations and efficacy. *Food Chem Toxicol* 43(2):187-201.
- Balkan, B.; Dunning, B.E. 1994. Glucosamine inhibits glucokinase in vitro and produces a glucosespecific impairment of in vivo insulin secretion in rats. *Diabetes* 43(10):1173-1179.
- Baron, A.D.; Zhu, J.-S.; Zhu, J.-H.; Weldon, H.; Maianu, L.; Garvey, W.T. 1995. Glucosamine induces insulin resistance in vivo by affecting GLUT 4 translocation in skeletal muscle. Implications for glucose toxicity. *J Clin Invest* 96(6):2792-2801.
- Biggee, B.A.; Blinn, C.M.; Nuite, M.; Silbert, J.E.; McAlindon, T.E. 2007. Effects of oral glucosamine sulphate on serum glucose and insulin during an oral glucose tolerance test of subjects with osteoarthritis. *Ann Rheum Dis* 66(2):260-262.
- Buse, M.G. 2006. Hexosamines, insulin resistance, and the complications of diabetes: current status. *Am J Physiol Endocrinol Metab* 290(1):E1-E8.
- Ciaraldi, T.P.; Carter, L.; Nikoulina, S.; Mudaliar, S.; McClain, D.A.; Henry, R.R. 1999. Glucosamine regulation of glucose metabolism in cultured human skeletal muscle cells: divergent effects on glucose transport/phosphorylation and glycogen synthase in non-diabetic and type 2 diabetic subjects. *Endocrinology* 140(9):3971-3980.
- Clegg, D.O.; Reda, D.J.; Harris, C.L.; Klein, M.A.; O'Dell, J.R.; Hooper, M.M.; Bradley, J.D.; Bingham, C.O. (3rd); Weisman, M.H.; Jackson, C.G.; Lane, N.E.; Cush, J.J.; Moreland, L.W.; Schumacher, H.R. (Jr.); Oddis, C.V.; Wolfe, F.; Molitor, J.A.; Yocum, D.E.; Schnitzer, T.J.; Furst, D.E.; Sawitzke, A.D.; Shi, H.; Brandt, K.D.; Moskowitz, R.W.; Williams, H.J. 2006. Glucosamine, chondroitin sulfate, and the two in combination for painful knee osteoarthritis. *N Engl J Med* 354(8):795-808.
- Crolle, G.; D'Este, E. 1980. Glucosamine sulphate for the management of arthrosis: a controlled clinical investigation. *Curr Med Res Opin* 7(2):104-109.

- D'Ambrosio, E.; Casa, B.; Bompani, R.; Scali, G.; Scali, M. 1981. Glucosamine sulphate: a controlled clinical investigation in arthrosis. *Pharmatherapeutica* 2(8):504-508.
- Das, A. (Jr.); Hammad, T.A. 2000. Efficacy of a combination of FCHG49(TM) glucosamine hydrochloride, TRH122(TM) low molecular weight sodium chondroitin sulfate and manganese ascorbate in the management of knee osteoarthritis. *Osteoarthritis Cartilage* 8(5):343-350.
- Drovanti, A.; Bignamini, A.A.; Rovati, A.L. 1980. Therapeutic activity of oral glucosamine sulfate in osteoarthritis: a placebo- controlled double-blind investigation. *Clin Ther* 3(4):260-272.
- Du, J.; White, N.; Eddington, N.D. 2004. The bioavailability and pharmacokinetics of glucosamine hydrochloride and chondroitin sulfate after oral and intravenous single dose administration in the horse. *Biopharm Drug Dispos* 25(3):109-116.
- Echard, B.W.; Talpur, N.A.; Funk, K.A.; Bagchi, D.; Preuss, H.G. 2001. Effects of oral glucosamine and chondroitin sulfate alone and in combination on the metabolism of SHR and SD rats. *Mol Cell Biochem* 225(1):85-91.
- Giordano, N.; Nardi, P.; Senesi, M.; Palumbo, F.; Battisti, E.; Gonnelli, S.; Franci, B.; Campagna, M.S.; Gennari, C. 1996. Efficacia e tollerabilità della glucosamina solfato nel trattamento della gonartrosi = The efficacy and safety of glucosamine sulfate in the treatment of gonarthrosis. *Clin Ter* 147(3):99-105.
- Grossman, S.H.; Dorn, C.G.; Potter, V.R. 1974. The preparation and characterization of pure rat liver glucokinase. *J Biol Chem* 249(10):3055-3060.
- Hawkins, M.; Barzilai, N.; Liu, R.; Hu, M.; Chen, W.; Rossetti, L. 1997. Role of the glucosamine pathway in fat-induced insulin resistance. *J Clin Invest* 99(9):2173-2182.
- Holmång, A.; Nilsson, C.; Niklasson, M.; Larsson, B.M.; Lonroth, P. 1999. Induction of insulin resistance by glucosamine reduces blood flow but not interstitial levels of either glucose or insulin. *Diabetes* ;48(1):106-111.
- Hughes, R.; Carr, A. 2002. A randomized, double-blind, placebo-controlled trial of glucosamine sulphate as an analgesic in osteoarthritis of the knee. *Rheumatology* 41(3):279-284.
- Kim, Y.B.; Zhu, J.S.; Zierath, J.R.; Shen, H.Q.; Baron, A.D.; Kahn, B.B. 1999. Glucosamine infusion in rats rapidly impairs insulin stimulation of phosphoinositide 3-kinase but does not alter activation of Akt/protein kinase B in skeletal muscle. *Diabetes* 48(2):310-320.
- Lafèrrière, B.; Garcia-Lorda, P.; Russell, C.D.; Pi-Sunyer, F.X. 2004. Effect of oral glucosamine sulfate on serum leptin levels in human subjects. *Nutrition* 20(3):321-322.
- Lavery, S.; Sandy, J.D.; Celeste, C.; Vachon, P.; Marier, J.F.; Plaas, A.H.K. 2005. Synovial fluid levels and serum pharmacokinetics in a large animal model following treatment with oral glucosamine at clinically relevant doses. *Arthritis Rheum* 52:181-191.

- Marshall, S.; Bacote, V.; Traxinger, R. 1991. Discovery of a metabolic pathway mediating glucose-induced desensitization of the glucose transport system. Role of hexosamine biosynthesis in the induction of insulin resistance. *J Biol Chem* 266(8):4706-4712.
- Marshall, S.; Nadeau, O.; Yamasaki, K. 2005. Glucosamine-induced activation of glycogen biosynthesis in isolated adipocytes. Evidence for a rapid allosteric control mechanism within the hexosamine biosynthesis pathway. *J Biol Chem* 280(12):11018-11024.
- McNamara, P.S.; Barr, S.C.; Erb, H.N. 1996. Hematologic, hemostatic, and biochemical effects in dogs receiving an oral chondroprotective agent for thirty days. *Am J Vet Res* 57(9):1390-1394.
- Meigs, J.B.; Nathan, D.M.; Wilson, P.W.F.; Cupples, L.A.; Singer, D.E. 1998. Metabolic risk factors worsen continuously across the spectrum of nondiabetic glucose tolerance: The Framingham Offspring Study. *Ann Intern Med* 128(7):524-533.
- Monauni, T.; Zenti, M.G.; Cretti, A.; Daniels, M.C.; Targher, G.; Caruso, B.; Caputo, M.; McClain, D.; Del Prato, S.; Giaccari, A.; Muggeo, M.; Bonora, E.; Bonadonna, R.C. 2000. Effects of glucosamine infusion on insulin secretion and insulin action in humans. *Diabetes* 49(6):926-935.
- Mueckler M. 1993. Glucokinase, glucose sensing, and diabetes. *Proc Natl Acad Sci USA* 90:784-785.
- Muniyappa, R.; Karne, R.J.; Hall, G.; Crandon, S.K.; Bronstein, J.A.; Ver, M.R.; Hortin, G.L.; Quon, M.J. 2006. Oral glucosamine for 6 weeks at standard doses does not cause or worsen insulin resistance or endothelial dysfunction in lean or obese subjects. *Diabetes* 55(11):3142-3150.
- Nelson, B.A.; Robinson, K.A.; Buse, M.G. 2000. High glucose and glucosamine induce insulin resistance via different mechanisms in 3T3-L1 adipocytes. *Diabetes* 49(6):981-991.
- Noack, W.; Fischer, M.; Forster, K.K.; Rovati, L.C.; Setnikar, I. 1994. Glucosamine sulfate in osteoarthritis of the knee. *Osteoarthritis Cartilage* 2(1):51-59.
- Oguchi, M.; Miyatake, Y.; Ayabe, J.; Akamatsu, N. 1975. Phosphorylation of D-glucosamine by rat liver glucokinase. *J Biochem (Tokyo)* 77(5):1117-1121.
- Oguchi, M.; Sato, M.; Miyatake, Y.; Akamatsu, N. 1977. Studies on the metabolism of hexosamine. A kinase activity for D-glucosamine without inhibition by glucose in rat liver and its possible identity with glucokinase. *J Biochem (Tokyo)* 82(2):559-567.
- Parry, M.J.; Walker, D.G. 1967. Further properties and possible mechanism of action of adenosine 5'-triphosphate-d-glucose 6-phosphotransferase from rat liver. *Biochem J* 105(2):473-482.
- Patti, M.E.; Virkamaki, A.; Landaker, E.J.; Kahn, C.R.; Yki-Jarvinen, H. 1999. Activation of the hexosamine pathway by glucosamine in vivo induces insulin resistance of early postreceptor insulin signaling events in skeletal muscle. *Diabetes* 48(8):1562-1571.

- Pavelká, K.; Gatterova, J.; Olejarova, M.; Machacek, S.; Giacobelli, G.; Rovati, L.C. 2002. Glucosamine sulfate use and delay of progression of knee osteoarthritis: a 3-year, randomized, placebo-controlled, double-blind study. *Arch Intern Med* 162(18):2113-2123.
- Persiani, S.; Roda, E.; Rovati, L.C.; Locatelli, M.; Giacobelli, G.; Roda, A. 2005. Glucosamine oral bioavailability and plasma pharmacokinetics after increasing doses of crystalline glucosamine sulfate in man. *Osteoarthritis Cartilage* 13(12):1041-1049.
- Pham, T.N.; Scofield, B.H. 2005. Glucosamine and insulin resistance. *J Investig Med* 53(1, Suppl. S):S271 [Abstract No. 104].
- Pouwels, M.-J.; Jacobs, J.R.; Span, P.N.; Lutterman, J.A.; Smits, P.; Tack, C.J. 2001. Short-term glucosamine infusion does not affect insulin sensitivity in humans. *J Clin Endocrinol Metab* 86(5):2099-2103.
- Pujalte, J.M.; Llavore, E.P.; Ylescupidéz, F.R. 1980. Double-blind clinical evaluation of oral glucosamine sulphate in the basic treatment of osteoarthritis. *Curr Med Res Opin* 7(2):110-114.
- Qiu, G.X.; Gao, S.N.; Giacobelli, G.; Rovati, L.; Setnikar, I. 1998. Efficacy and safety of glucosamine sulphate versus ibuprofen in patients with knee osteoarthritis. *Arzneimittel-Forschung* 48(5):469-474.
- Reginster, J.Y.; Deroisy, R.; Rovati, L.C.; Lee, R.L.; Lejeune, E.; Bruyere, O.; Giacobelli, G.; Henrotin, Y.; Dacre, J.E.; Gossett, C. 2001. Long-term effects of glucosamine sulphate on osteoarthritis progression: a randomised, placebo-controlled clinical trial. *Lancet* 357(9252):251-256.
- Robertson, M.W.; Dunshea, F.R.; Goddard, M.E.; Leury, B.J. 2005. Stimulation of the hexosamine biosynthetic pathway by glucosamine in sheep. *Asia Pac J Clin Nutr* 14(Suppl.):S79.
- Roda, A.; Sabatini, L.; Barbieri, A.; Guardigli, M.; Locatelli, M.; Violante, F.S.; Rovati, L.C.; Persiani, S. 2006. Development and validation of a sensitive HPLC-ESI-MS/MS method for the direct determination of glucosamine in human plasma. *J Chromatogr B* 844(1):119-126.
- Rossetti, L.; Hawkins, M.; Chen, W.; Gindi, J.; Barzilai, N. 1995. In vivo glucosamine infusion induces insulin resistance in normoglycaemic but not in hyperglycaemic conscious rats. *J Clin Invest* 96(1):132-140.
- Rovati, L.C. 1992. Clinical research in osteoarthritis: Design and results of short-term and long-term with disease-modifying drugs. *Int J Tissue React* 14(5):243-251.
- Sakai, K.; Clemmons, D.R. 2003. Glucosamine induces resistance to insulin-like growth factor I (IGF-I) and insulin in Hep G2 cell cultures: Biological significance of IGF-I/insulin hybrid receptors. *Endocrinology* 144(6):2388-2395.
- Salyers, A.A.; Vercellotti, J.R.; West, S.E.H.; Wilkins, T.D. 1977. Fermentation of mucin and plant polysaccharides by strains of bacteroids from human colon. *Appl Environ Microbiol* 33(2):319-22.

- Scroggie, D.A.; Albright, A.; Harris, M.D. 2003. The effect of glucosamine-chondroitin supplementation on glycosylated haemoglobin levels in patients with type 2 diabetes mellitus: a placebo-controlled, double-blinded, randomized clinical trial. *Arch Intern Med* 163(13):1587-1590.
- Setnikar, I.; Rovati, L.C. 2001. Absorption, distribution, metabolism and excretion of glucosamine sulfate. A review. *Arzneimittelforschung* 51(9):699-725.
- Setnikar, I.; Pacini, M.A.; Revel, L. 1991. Antiarthritic effects of glucosamine sulfate studied in animal models. *Arzneimittelforschung* 41(5):542-545.
- Setnikar, I.; Palumbo, R.; Canali, S.; Zanolò, G. 1993. Pharmacokinetics of glucosamine in man. *Arzneimittelforschung* 43(10):1109-1113.
- Spampinato, D.; Giaccari, A.; Trischitta, V.; Costanzo, B.V.; Morviducci, L.; Buongiorno, A.; Di, Mario, U.; Vigneri, R.; Frittitta, L. 2003. Rats that are made insulin resistant by glucosamine treatment have impaired skeletal muscle insulin receptor phosphorylation. *Metabolism* 52(9):1092-1095.
- Stender, S.; Astrup, P. 1977. Glucosamine and experimental atherosclerosis: Increased wet weight and changed composition of cholesterol fatty acids in aorta of rabbits fed a cholesterol-enriched diet with added glucosamine. *Atherosclerosis* 26(2):205-213.
- Storer, A.C.; Cornish-Bowden, A. 1976. Kinetics of rat liver glucokinase. Co-operative interactions with glucose at physiologically significant concentrations. *Biochem J* 159(1):7-14.
- Stumpf, J.L.; Lin, S.W. 2006. Effect of glucosamine on glucose control. *Ann Pharmacother* 40:69-698.
- Tannis, A.J.; Barban, J.; Conquer, J.A. 2004. Effect of glucosamine supplementation on fasting and non-fasting plasma glucose and serum insulin concentrations in healthy individuals. *Osteoarthritis Cartilage* 12(6):506-511.
- Tapadinhas, M.J.; Rivera, I.C.; Bignamini, A.A. 1982. Oral glucosamine sulphate in the management of arthrosis: report on a multi- centre open investigation in Portugal. *Pharmatherapeutica* 3(3):157-168.
- Virkamäki, A.; Daniels, M.C.; Hämaläinen, S.; Utriainen, T.; McClain, D.; Yki-Järvinen, H. 1997. Activation of the hexosamine pathway by glucosamine in vivo induces insulin resistance in multiple insulin sensitive tissues. *Endocrinology* 138(6):2501-2507.
- Weiden, S.; Wood, I.J. 1958. The fate of glucosamine hydrochloride injected intravenously in man. *J Clin Pathol* 11(4):343-349.
- Xu, L.Z.; Weber, I.T.; Harrison, R.W.; Gidh-Jain, M.; Pilkis, S.J. 1995. Sugar specificity of human beta-cell glucokinase: correlation of molecular models with kinetic measurements. *Biochemistry* 34(18):6083-6092.
- Yu, J.G.; Boies, S.M.; Olefsky, J.M. 2003. The effect of oral glucosamine sulfate on insulin sensitivity in human subjects. *Diabetes Care* 26(6):1941-1942.

APPENDIX A

Summary of Clinical Studies

APPENDIX A**Summary of Clinical Studies**

Criteria for Determining the Study Quality	
Levels of Evidence	Type of Evidence from Human Studies
I	Well-designed, systematic reviews and meta-analyses of randomized controlled trials or other clinical trials; well-designed, randomized, controlled trials
II	Well-designed clinical trials without randomization and/or control groups
III	Well-designed descriptive and observational studies (e.g., correlational studies, cohort studies, case-control studies)
IV	Peer-reviewed published articles; Conclusions of other reputable regulatory agencies; Previous marketing experience; Expert opinion reports
V	Abstracts only

Table A-1 Clinical Studies Reporting Glucosamine Administration, Containing Indices and Glucose and Insulin Homeostasis							
Source	Study Objective and Design	GlcN Dose and Duration	Fasting Glucose	Glucose Challenge	Insulin Levels/ Sensitivity	Conclusions and Other Relevant Observations	Study Quality Rating
Biggee <i>et al.</i> , 2007	<p>Objective: Determine effect of GlcN on serum Glc and insulin</p> <p>Subjects: N = 16 13 normoglycaemic 3 unsuspected diabetics 11 women, 5 men (41-74 yrs; 42-132 kg)</p> <p>Relevant Exclusions: Diabetics; subjects with altered glucose metabolism.</p> <p>Design: Study performed over 3 visits 1-2 weeks apart.</p> <p>Sera from 16 osteoarthritis patients analyzed for Glc and Insulin at fasting and during a 3-hr time interval following a single GlcN, Glucose, and Glucose + GlcN dose.</p>	1,500 mg Glucosamine sulphate	<p>Baseline: values not reported</p> <p>Treatment: GlcN had no effect on fasting Glucose levels.</p>	<p>Normoglycaemic Individuals: GlcN resulted in a 34% increase in AUC (P=NS) (29±27 vs. 39±22) (Control vs. GlcN) (mg min/ml)</p>	<p>Normoglycaemic Individuals: GlcN did Not affect insulin levels during glucose challenge (7.6±4.1 vs. 7.4±4.2) (Control vs. GlcN) (mIU min/ml)</p>	<p>GlcN consumption immediately prior to glucose challenge did not affect glucose or insulin metabolism in non-diabetic non-glucose intolerant subjects.</p> <p>Authors concluded that In unsuspected diabetics GlcN exacerbated the glucose intolerance response.</p>	III
				<p>Unsuspected Diabetics: GlcN resulted in a 32% in AUC (P=0.04) (145±86 vs. 191±94) (Control vs. GlcN) (mg/min/ml)</p>	<p>Unsuspected Diabetics: Blunted insulin response following glucose challenge. (8.9±3.8 vs. 8.5±3.3) (Control vs. GlcN) (mIU min/ml)</p>		

Table A-1 Clinical Studies Reporting Glucosamine Administration, Containing Indices and Glucose and Insulin Homeostasis							
Source	Study Objective and Design	GlcN Dose and Duration	Fasting Glucose	Glucose Challenge	Insulin Levels/ Sensitivity	Conclusions and Other Relevant Observations	Study Quality Rating
Muniyappa <i>et al.</i> , 2006	<p>Objective: To Determine the effect of GlcN on insulin resistance or endothelial dysfunction in lean or obese subjects.</p> <p>Subjects: Healthy subjects (22-65 yrs) 32 lean (=25 kg/m²) 52 obese (=30 kg/m²)</p> <p>Relevant Exclusions: Diabetics</p> <p>Design: Randomized double blind placebo-controlled crossover study.</p> <p>Subjects received GlcN or placebo for 6 weeks followed by a 1-week washout and 6 weeks of opposite treatment.</p>	<p>1,00 mg Glucosamine hydrochloride</p> <p>500 mg t.i.d. for 6 weeks</p>	<p>Baseline values were reported (no differences).</p> <p>6 weeks of GlcN had no effect on fasting Glc levels in lean or obese subjects.</p> <p>Lean: (83±2 vs. 82±1) (Control vs. GlcN) (mg/dl)</p> <p>Obese: (87±2 vs. 87±2) (Control vs. GlcN) (mg/dl)</p>		<p>6 weeks of GlcN had no effect on fasting insulin levels in lean or obese subjects.</p> <p>Lean: (5.0±0.7 vs. 4.4±0.5) (Control vs. GlcN) (µU/ml)</p> <p>Insulin sensitivity measured by Glc clamp method was not significantly different between groups: (6.9 vs. 7.6) (Control vs. GlcN) (10⁻⁴ dl/kg min)</p> <p>Obese: (11.4±1.3 vs. 11.3±1.8) (Control vs. GlcN) (mg/dl)</p> <p>Insulin sensitivity measured by Glc clamp method was not significantly different between groups: (5.4 vs. 4.1) (10⁻⁴ dl/kg min)</p>	6 weeks of daily oral GlcN did not cause insulin resistance or endothelial cell dysfunction in lean or obese healthy subjects.	I

Table A-1 Clinical Studies Reporting Glucosamine Administration, Containing Indices and Glucose and Insulin Homeostasis							
Source	Study Objective and Design	GlcN Dose and Duration	Fasting Glucose	Glucose Challenge	Insulin Levels/ Sensitivity	Conclusions and Other Relevant Observations	Study Quality Rating
Clegg <i>et al.</i> , 2006	<p>Objective: To determine the efficacy of GlcN, Chondroitin sulphate (CS), and the 2 in combination for painful knee osteoarthritis.</p> <p>Subjects: N = 1,583 (58±10 yrs) BMI ~ 32</p> <p>Relevant exclusions: Diabetics or subjects with impaired glucose metabolism were NOT excluded; however number not indicated.</p> <p>Method: Randomized placebo controlled, double-blind, multicentre intervention trial sponsored by NIH.</p> <p>Subjects were allocated to 6 months of control, GlcN, GlcN + CS, Celoxecib, or placebo treatment.</p> <p>Primary endpoint was WOMAC pain score.</p> <p>Patients with diabetes had</p>	<p>1,500 mg Glucosamine hydrochloride</p> <p>500 mg t.i.d. for 6 months</p>	No difference, data not reported.			<p>In the methods section, the study states that medication was withdrawn in patients in whom diabetes or G.I. bleeding developed, and patients were referred for further evaluation. However, no further details on the incidence of diabetes in the treatment groups were reported.</p> <p>No increase risk of cardiovascular disease in diabetics receiving GlcN.</p>	I

Table A-1 Clinical Studies Reporting Glucosamine Administration, Containing Indices and Glucose and Insulin Homeostasis							
Source	Study Objective and Design	GlcN Dose and Duration	Fasting Glucose	Glucose Challenge	Insulin Levels/ Sensitivity	Conclusions and Other Relevant Observations	Study Quality Rating
	fasting blood glucose, or glycosylated haemoglobin levels monitored during study.						
Pham and Scofield, 2005 *Abstract*	<p>Objective: To determine whether insulin resistance occurs in non-diabetic subjects after 6 weeks of GlcN.</p> <p>Subjects: 32 (No details reported)</p> <p>Relevant Exclusions: Diabetics; subjects on medication known to cause insulin resistance.</p> <p>Methods: Prospective, study without control groups.</p> <p>All subjects had evaluations of fasting insulin and glucose values before and after 6 weeks of GlcN treatment.</p>	<p>1500 mg Glucosamine</p> <p>6 weeks</p> <p>Route of administration not reported</p>	Data not reported		<p>Log HOMA values: increased 25% following GlcN treatment (P<0.008)</p> <p>(0.29±37 vs. 0.36±36) (baseline vs. week 6)</p> <p>QUIKI values: Decreased by 5% following GlcN treatment (P=0.017)</p> <p>0.64±0.13 vs. 0.61±0.12 (baseline vs. week 6)</p>	<p>Small and large artery elasticity worsened, decreasing by ~8% (P = NS).</p> <p>Authors concluded that 6 weeks of GlcN causes insulin resistance in humans.</p>	V

Table A-1 Clinical Studies Reporting Glucosamine Administration, Containing Indices and Glucose and Insulin Homeostasis							
Source	Study Objective and Design	GlcN Dose and Duration	Fasting Glucose	Glucose Challenge	Insulin Levels/ Sensitivity	Conclusions and Other Relevant Observations	Study Quality Rating
Tannis <i>et al.</i> 2004	<p>Objective: To determine the effect of GlcN on fasting and non-fasting plasma glucose and serum insulin in healthy individuals.</p> <p>Subjects: Healthy male and female adults (n=19) (22-58 years) (BMI: 18.6-31.1)</p> <p>Relevant Exclusions: Subjects using medication that may interfere with glucose metabolism; BMI<18 or >32; diabetics</p> <p>Methods: Double blind placebo controlled supplementation trial. Patients were randomized to receive 500 mg of GlcN or placebo (dextrose) t.i.d. for 12 weeks. Oral glucose tolerance tests were conducted at baseline and after an overnight fast on weeks 6 and 12.</p>	<p>1,500 mg Glucosamine Sulphate</p> <p>12 weeks</p>	<p>Baseline glucose AUC revealed no differences between groups.</p> <p>No significant differences in fasting blood glucose levels between groups at week 6 or 12.</p> <p>Data reported in graphical form, no tabulated values.</p>	<p>No significant difference in plasma glucose response before, during and after GlcN use.</p> <p>Week 0: 4.24±0.73 vs. 4.57±0.55 (control vs. GlcN) (mmol/L)</p> <p>Week 6: 4.52±0.65 vs. 3.85±0.20 (control vs. GlcN) (mmol/L)</p> <p>Week 12: 4.47±0.84 vs. 4.40±0.63 (control vs. GlcN) (mmol/L)</p>	<p>Fasting insulin levels did not differ between groups.</p> <p>Week 0: 4.68±1.4 vs. 5.69±1.7 (control vs. GlcN) (µIU/ml)</p> <p>Week 6: 3.38±2.6 vs. 4.96±4.7 (control vs. GlcN) (µIU/ml)</p> <p>Week 12: 6.8±3.4 vs. 7.7±3.8 (control vs. GlcN) (µIU/ml)</p>	<p>No significant change in glycated haemoglobin levels at baseline, week 6 and week 12.</p> <p>GlcN use does not cause intolerance in healthy adults.</p>	I

Table A-1 Clinical Studies Reporting Glucosamine Administration, Containing Indices and Glucose and Insulin Homeostasis							
Source	Study Objective and Design	GlcN Dose and Duration	Fasting Glucose	Glucose Challenge	Insulin Levels/ Sensitivity	Conclusions and Other Relevant Observations	Study Quality Rating
Lafèrriere <i>et al.</i> , 2004	<p>Objective: To determine the effect of oral GlcN on serum leptin levels</p> <p>Subjects: 20 healthy non-obese subjects with normal glucose tolerance.</p> <p>BMI 20.8 – 29.5 kg/m² 18-35 years</p> <p>Relevant Exclusions: Not reported</p> <p>Methods: 6 subjects received 3,000 mg of Glucosamine, and 5 received 6,000 mg of glucosamine in the morning. At a subsequent visit subjects received the same dose of GlcN in conjunction with dexamethasone infusion. 9 control subjects were used under the same conditions.</p> <p>Plasma insulin and glucose was monitored after an overnight fast, and after another 9 hrs of fasting.</p>	<p>3,000 mg or 6,000 mg</p> <p>GlcN sulphate Single dose (acute)</p>	<p>Plasma glucose following 9 hrs of fasting did not change with GlcN administration (data not reported).</p>			<p>9-hr leptin levels dropped by 24±6% in controls; 28±15% in the 3,000 mg group; and by 40±5% in the 6,000 mg group. The differences were not significant. No synergy with dexamethasone was observed.</p> <p>Authors concluded that acute high dose oral GlcN does not affect serum leptin, or modify glucose or insulin levels in humans.</p>	II

Table A-1 Clinical Studies Reporting Glucosamine Administration, Containing Indices and Glucose and Insulin Homeostasis							
Source	Study Objective and Design	GlcN Dose and Duration	Fasting Glucose	Glucose Challenge	Insulin Levels/ Sensitivity	Conclusions and Other Relevant Observations	Study Quality Rating
Yu <i>et al.</i> , 2003	<p>Objective: To determine the effect of GlcN on insulin sensitivity</p> <p>Subjects: 7 obese: (BMI =27 kg/m²) 7 lean: (BMI =27 kg/m²)</p> <p>3 obese and 2 lean subjects displayed impaired Glc tolerance.</p> <p>Relevant Exclusions: None stated</p> <p>Methods: Subjects received GlcN 500 mg t.i.d. for 4 wks, and measurements of meal tolerance and glucose tolerance measured before and after GlcN use.</p>	<p>1,500 mg Glucosamine Sulphate</p> <p>4 weeks</p>	<p>Baseline: 5.4±0.3 vs. 4.8±0.3 (obese vs. lean) (mmol/L)</p> <p>No differences in fasting glucose after 4 wks of GlcN (data not reported).</p>	<p>AUC_{gluc} all subjects: 1,551±55 vs. 1,539±55 (baseline vs. wk 4) (mmol/L min)</p> <p>AUC_{gluc} Obese vs. Lean not reported.</p>	<p>Baseline: 14.4±3.6 vs. 10.8±4.9 (obese vs. lean) (µU/ml)</p> <p>No differences in fasting insulin after 4 wks of GlcN (data not shown)</p> <p>AUC_{insulin} Baseline: 35,800 vs. 12,000 (lean vs. obese) (P<0.01). (mµ/L min)</p> <p>AUC_{insulin} All Subjects: 17,903 vs. 17,861 (baseline vs. wk 4) (mµ/L min)</p> <p>Insulin Sensitivity: 2.37±0.46 vs. 2.55±0.58 (Baseline vs. wk 4)</p> <p>No difference in insulin sensitivity when analyzed according to BMI or glucose tolerance.</p>	<p>No effect of GlcN on insulin sensitivity in obese vs. lean, glucose intolerant vs. glucose tolerant or in total group analysis at wk 4 vs. baseline.</p>	II

Table A-1 Clinical Studies Reporting Glucosamine Administration, Containing Indices and Glucose and Insulin Homeostasis							
Source	Study Objective and Design	GlcN Dose and Duration	Fasting Glucose	Glucose Challenge	Insulin Levels/ Sensitivity	Conclusions and Other Relevant Observations	Study Quality Rating
Scroggie <i>et al.</i> , 2003	<p>Objective: To determine the effect of GlcN on glycosylated haemoglobin in type II diabetics.</p> <p>Subjects: 38 male and female (26 treatment to 12 placebo) GlcN (68.6 yrs) Placebo (70.7 yrs)</p> <p>Relevant exclusions: Patients taking insulin, with unstable blood Glc levels, recently diagnosed patients</p> <p>Methods: Placebo controlled, randomized, double blinded trial.</p> <p>Subjects received glucosamine + chondroitin sulphate daily for 90 days.</p> <p>Baseline and 90-day plasma samples analyzed.</p>	<p>1,500 mg glucosamine 500 mg t.i.d.</p> <p>+</p> <p>1,200 mg Chondroitin 400 mg t.i.d.</p> <p>90 days</p>				<p>4 drop-outs in the treatment group were reported as not due to GlcN or worsening of glycaemic control.</p> <p>Haemoglobin A_{1c} levels were increased by 0.5% and 0.16% in the GlcN and placebo groups respectively at end of the study relative to baseline levels (P= NS between groups or baseline).</p> <p>HbA1c levels: GlcN: (6.45 vs. 6.5) (Baseline vs. day 90)</p> <p>Placebo: (6.25 vs. 6.09) (Baseline vs. day 90)</p>	I

Table A-1 Clinical Studies Reporting Glucosamine Administration, Containing Indices and Glucose and Insulin Homeostasis							
Source	Study Objective and Design	GlcN Dose and Duration	Fasting Glucose	Glucose Challenge	Insulin Levels/ Sensitivity	Conclusions and Other Relevant Observations	Study Quality Rating
Pavelká <i>et al.</i> , 2002	<p>Objective: To determine the effect of glucosamine sulphate on the delay of progression of knee osteoarthritis</p> <p>Subjects: 202 patients with knee osteoarthritis 70-80% women ~60+7 yrs; ~25±2 kg/m²</p> <p>Relevant Exclusions: Metabolic disorders or history of diabetes mellitus.</p> <p>Methods: 202 patients randomized to receive GlcN sulphate or placebo, 1500 mg once daily. Primary endpoint was change in joint space width and Lequesne and WOMAC score.</p>	<p>1,500 mg Glucosamine sulphate</p> <p>Once daily</p> <p>3 years</p>				<p>4 patients developed clinically evident diabetes during the study 3 in the placebo group and 1 in the GlcN group.</p> <p>The authors concluded that long-term GlcN treatment reduced the progression of knee osteoarthritis.</p>	I
Pouwels <i>et al.</i> , 2001	<p>Objective: To determine the short-term effect of glucosamine infusion on insulin sensitivity in humans.</p> <p>Subjects: 20 normoglycaemic healthy volunteers</p>	<p>1 µmol/dL min</p> <p>Glucosamine infusion for 300 min</p> <p>Resulting venous blood concentration</p>			<p>GlcN had no effect on insulin sensitivity.</p> <p>Glucose infusion rates during last 90 min of clamp were: (57±4 vs. 50±7) (µmol/kg min) (Placebo vs. GlcN)</p>	<p>Short-term GlcN infusion does affect insulin sensitivity.</p>	II

Table A-1 Clinical Studies Reporting Glucosamine Administration, Containing Indices and Glucose and Insulin Homeostasis							
Source	Study Objective and Design	GlcN Dose and Duration	Fasting Glucose	Glucose Challenge	Insulin Levels/ Sensitivity	Conclusions and Other Relevant Observations	Study Quality Rating
	<p>(10 men: 10 women) (24±4 yrs; 22.3±1.9 kg/m²).</p> <p>Relevant Exclusions: History of diabetes mellitus.</p> <p>Methods: Three protocols using the hyperinsulinaemic, euglycaemic clamp technique over a 300-minute interval. 1. Control = Saline infusion 2. GlcN₁₅₀ = GlcN infusion from 90-240 min (150 min). 3. GlcN₃₀₀ = GlcN infusion from 0-300 min.</p>	[0.15 mmol]			<p>GlcN infusion had no effect on forearm blood flow.</p> <p>GlcN had no affect on forearm arterial-venous blood glucose difference</p> <p>GlcN had no effect on forearm glucose uptake: 2.88±0.83 vs. 3.35±1.66 (GlcN_{150min} vs. control) (µmol/dL min) (P= NS)</p> <p>2.77±0.70 vs. 2.08±0.33 (GlcN_{300min} vs. control) (µmol/dL min).</p>		
Reginster <i>et al.</i> , 2001	<p>Objective: To determine the effect of long-term GlcN administration on osteoarthritis progression.</p> <p>Subjects: 212 with primary knee osteoarthritis. >50 yrs; ~27±2.5 kg/m²</p> <p>Relevant Exclusions: Subjects with substantial abnormalities in metabolic functions.</p>	<p>1,500 mg</p> <p>Glucosamine sulphate</p> <p>Once daily</p> <p>3 years</p>				<p>Drop-out rate in GlcN and placebo were equal (36 vs. 33%; P= 0.77), and no differences in drop-out reasons were noted.</p> <p>Routine laboratory monitoring did not show any changes in glycaemic homeostasis, with fasting plasma Glc levels decreasing slightly for the GlcN group relative to placebo (Data</p>	I

Table A-1 Clinical Studies Reporting Glucosamine Administration, Containing Indices and Glucose and Insulin Homeostasis							
Source	Study Objective and Design	GlcN Dose and Duration	Fasting Glucose	Glucose Challenge	Insulin Levels/ Sensitivity	Conclusions and Other Relevant Observations	Study Quality Rating
	<p>Methods: Patients were randomized to receive placebo or GlcN treatment once daily for 3 years. Primary endpoint was mean joint space width Secondary outcome included safety monitoring and testing of glucose homeostasis assessed by fasting glucose concentrations at yearly intervals.</p>					not reported).	
Monauni <i>et al.</i> , 2000	<p>Objectives: To determine the effects of glucosamine infusion on insulin action.</p> <p>Subjects: 10 healthy male volunteers. (24.9±0.43 yrs; 23.5±0.81 kg/m²) No family history of diabetes and all subjects had normal oral glucose tolerance tests.</p> <p>Relevant Exclusions: None reported.</p> <p>Methods: Subjects participated in 2 or 3 performed at random 2-3</p>	<p>1.6 µmol/min kg (low GlcN)</p> <p>5 µmol/min kg (high GlcN)</p> <p>Infusion</p>	<p>Fasting glucose modestly increased following GlcN infusion.</p> <p>Saline: (5.2±0.02 mmol/L)</p> <p>Low GlcN: (5.3±0.02 mmol/L) (P<0.02 vs. saline)</p> <p>High GlcN: (5.5±0.01 mmol/L) (P<0.05 vs.</p>	<p>Glucose levels increased slightly (P<0.01) in the high GlcN vs. the control, no difference between low GlcN vs. control.</p> <p>Tritiated glucose levels increase slightly for high GlcN group vs. controls (P<0.01).</p>	<p>Fasting insulin levels not affected by GlcN.</p> <p>Saline: (59±5.2 pmol/L)</p> <p>Low GlcN: (55±3.5 pmol/L)</p> <p>High GlcN: (47.9 ± 1.6 pmol/L)</p> <p>Glucose Tolerance: Insulin levels not affected by GlcN during the glucose tolerance test.</p> <p>No significant</p>	<p>Plasma GlcN concentrations increased to a maximum of 0.57±0.14 mmol/L and 1.15±0.18 in the low ad high GlcN treatment groups respectively vs. 0.04 ±0.007 in saline group.</p> <p>Authors concluded that at high GlcN infusion doses that GlcN can impair glucose intolerance under hyperglycaemic/ hyperinsulinaemic conditions and decrease glucose effectiveness at</p>	II

Table A-1 Clinical Studies Reporting Glucosamine Administration, Containing Indices and Glucose and Insulin Homeostasis							
Source	Study Objective and Design	GlcN Dose and Duration	Fasting Glucose	Glucose Challenge	Insulin Levels/ Sensitivity	Conclusions and Other Relevant Observations	Study Quality Rating
	<p>wks apart.</p> <p>Catheter inserted into wrist vein.</p> <p>Starting at time = -60 min subjects received a continuous infusion of saline (saline study n=10), or GlcN at a rate of 1.6 $\mu\text{mol}/\text{min kg}$ (low GlcN study n=10), or GlcN at a rate of 5 $\mu\text{mol}/\text{min kg}$ (high GlcN study n=5).</p> <p>At t = 0, a glucose tolerance test was performed. At 180 min, a euglycaemic insulin clamp was carried out for 120 min.</p> <p>Studies were performed after an overnight fast.</p>		saline)		<p>differences or trends between readily releasable insulin, and glucose stimulated insulin secretion among groups.</p> <p>Plasma glucose threshold of insulin secretion was increased in both GlcN groups relative to controls (~10%; P<0.05).</p> <p>Insulin sensitivity (S1) under hyperglycaemic/ hyperinsulinaemic conditions was significantly blunted by 30% (P<0.01) following high GlcN infusion.</p> <p>Glucose effectiveness (Sg) at basal steady state insulinaemia was blunted by 40% (P<0.05).</p> <p>Insulin Clamp: Whole body glucose utilization and endogenous glucose output (EGO) during systemic hyperinsulinaemia were</p>	<p>basal steady state insulinaemia. The net result of which is a mild disruption of glucose homeostasis.</p> <p>However no GlcN induced effects were observed under euglycaemic conditions.</p>	

Table A-1 Clinical Studies Reporting Glucosamine Administration, Containing Indices and Glucose and Insulin Homeostasis							
Source	Study Objective and Design	GlcN Dose and Duration	Fasting Glucose	Glucose Challenge	Insulin Levels/ Sensitivity	Conclusions and Other Relevant Observations	Study Quality Rating
					<p>unaffected by GlcN infusion.</p> <p>Rates of glucose oxidation and glucose storage were unaffected by GlcN.</p>		
<p>Almada <i>et al.</i>, 2000</p> <p>*Abstract*</p>	<p>Objective: To determine the effect of GlcN sulphate on chronic lower back pain.</p> <p>Subjects: 15 non-diabetic subjects (47.9±11.3 yrs; 27.2±4.4 kg/m²)</p> <p>Relevant Exclusions: Not reported</p> <p>Methods: Double-blind, placebo controlled, trial.</p> <p>Subjects randomized to receive 500 mg of GlcN t.i.d. or placebo for 12 weeks.</p>	<p>1,500 mg Glucosamine Sulphate</p> <p>500 mg t.i.d.</p> <p>12 weeks</p>	<p>Baseline: (5.2±0.5 vs. 5.2±0.9) (placebo vs. GlcN)</p> <p>Week-12: (5.2±0.7 vs. 5.1±0.7) (placebo vs. GlcN)</p>		<p>Baseline: (17.9±10.1 vs. 19.2±10.8) (Placebo vs. GlcN)</p> <p>Week-12: No effect on fasting insulin levels. (16.8±10.1 vs. 23.9±12.7) (placebo vs. GlcN)</p> <p>Relative change (?) in fasting insulin from baseline was significantly increased between groups.</p> <p>(-1.1±3.8 vs. 4.9±4.3) (placebo vs. GlcN) (P<0.01)</p>	<p>Authors concluded additional studies using more rigorous methods to assess insulin resistance are warranted.</p>	V

Table A-1 Clinical Studies Reporting Glucosamine Administration, Containing Indices and Glucose and Insulin Homeostasis							
Source	Study Objective and Design	GlcN Dose and Duration	Fasting Glucose	Glucose Challenge	Insulin Levels/ Sensitivity	Conclusions and Other Relevant Observations	Study Quality Rating
Das and Hammad, 2000	<p>Objective: To determine the efficacy of glucosamine hydrochloride, chondroitin sulphate, and manganese ascorbate in the treatment of knee osteoarthritis.</p> <p>Subjects: 93 patients with knee osteoarthritis GlcN: (n=46; 64.59.8 yrs; 30.5±1.0 kg/m²) Placebo (n=47; 66.0±1.5 yrs; 30.2 kg/m²)</p> <p>Relevant exclusions: Subjects with non-insulin dependent diabetes; subjects with metabolic diseases.</p> <p>Methods: Randomized, double-blinded, placebo controlled study. 93 subjects were randomized to receive treatment or placebo twice daily for 6 months. Patients were evaluated at baseline, and every 2 months for management of knee osteoarthritis.</p>	<p>1000 mg</p> <p>Glucosamine hydrochloride</p> <p>6 months</p>	Baseline fasting glucose levels not measured.			<p>One patient in the GlcN group developed type II diabetes. Authors did not measure baseline fasting glucose levels and could not determine in the development of type II diabetes was treatment related.</p> <p>Authors concluded that glucosamine was effective in management of knee osteoarthritis.</p>	I

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Source	Study Objective and Design	GlcN Dose and Duration	Fasting Glucose	Glucose Challenge	Insulin Levels/ Sensitivity	Conclusions and Other Relevant Observations	Study Quality Rating
Tapadinhas <i>et al.</i> , 1982	<p>Objective: To assess the effectiveness and tolerability of oral glucosamine sulphate in the treatment of arthrosis.</p> <p>Subjects: 516 males, 692 females, ranging in age from 16 to 84 yrs (54.2±9.36 yrs); all patients suffered from arthrosis. Of the 1,208 patients, 92 subjects had diabetes and 74 subjects were using hypoglycaemic medication.</p> <p>Relevant exclusions: Not reported.</p> <p>Methods: Observational prospective study of 1,208 patients under the supervision of 252 doctors throughout Portugal. Subjects received 500 mg of GlcN sulphate t.i.d., for 6-8 weeks.</p>	<p>1,500 mg Glucosamine Sulphate</p> <p>500 mg t.i.d.</p> <p>6-8 weeks</p>				<p>92 diabetics and 74 patients receiving hypoglycaemic medication were included in the study.</p> <p>Authors reported that, no variation in tolerability was observed in the presence diabetes or with the treatment of hypoglycaemic medication.</p> <p>Authors concluded that oral GlcN was well-tolerated and effective in treating the symptoms of arthrosis.</p>	II

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Source	Study Objective and Design	GlcN Dose and Duration	Fasting Glucose	Glucose Challenge	Insulin Levels/ Sensitivity	Conclusions and Other Relevant Observations	Study Quality Rating
D'Ambrosio <i>et al.</i> , 1981	<p>Objective: To determine the efficacy and tolerance of GlcN sulphate in patients with osteoarthritis.</p> <p>Subjects: 30 patients with chronic degenerative osteoarthrosic disorders.</p> <p>GlcN: (3 male 12 female; 74.2±0.6 yrs) Control: (4 male, 11 female; 75.1±2.4 yrs)</p> <p>Relevant Exclusions: Not reported</p> <p>Methods: Patients were randomized to control or GlcN groups receiving</p>	<p>400 mg glucosamine sulphate</p> <p>Intramuscular intraarticular, or intravenous injection daily for 7 days</p> <p>Followed by</p> <p>500 mg t.i.d. glucosamine sulphate for an additional 14 days.</p>	<p>Baseline: 1.09±0.6 vs. 1.04±0.05 (GlcN vs. Control) (g/L)</p> <p>Day 14: 0.97±0.05 vs. 0.96 ±0.03 (GlcN vs. Control) (g/L)</p>			GlcN is effective and well tolerated for the treatment of arthrosis.	I
Drovanti <i>et al.</i> , 1980	<p>Objective: To determine the therapeutic activity of glucosamine sulphate in osteoarthritis.</p> <p>Subjects: 80 patients GlcN: (61.3±2.1 yrs) Placebo: (58.7±2.5 yrs)</p>	<p>1,500 mg Glucosamine sulphate</p> <p>500 mg t.i.d</p> <p>30 days</p>	<p>Baseline: 0.82±0.02 vs. 0.79±0.03 (GlcN vs. Control) (g/L)</p> <p>Day 30: 0.82±0.02 vs. 0.79 ±0.03 (GlcN vs. Control) (g/L)</p>				

Table A-1 Clinical Studies Reporting Glucosamine Administration, Containing Indices and Glucose and Insulin Homeostasis							
Source	Study Objective and Design	GlcN Dose and Duration	Fasting Glucose	Glucose Challenge	Insulin Levels/ Sensitivity	Conclusions and Other Relevant Observations	Study Quality Rating
	<p>Relevant Exclusions: Not reported</p> <p>Methods: 80 patients randomized to receive GlcN or placebo treatment. Efficacy monitored at days 7, 14, 21, and 30. Clinical safety assessed at baseline and at end of study.</p>						
Crolle and D'Este, 1980	<p>Objective: To determine the efficacy of parenteral administration of Glucosamine compared with parenteral administration of a reference drug, and oral GlcN treatment vs. oral placebo.</p> <p>Subjects: 40 in patients with chronic arthrosis. GlcN: 15 (7 male and 8 female; 70.5±3.2 yrs) Placebo: 15 (1 male, 14 females; 74.9±2.6 yrs)</p> <p>Relevant Exclusions: Not reported.</p> <p>Methods: 15 patients received 1 intramuscular injection daily</p>	<p>400 mg Glucosamine Sulphate</p> <p>Intramuscular injection for 7 days</p> <p>Followed by 1,500 mg</p> <p>Oral Glucosamine sulphate</p> <p>500 mg t.i.d. for 14 days</p>	<p>Baseline: 5.26±1.56 vs. 4.71±0.07 (GlcN vs. Placebo) (mmol/L)</p> <p>Day-21: 5.70±1.27 vs. 5.43±1.33 (GlcN vs. Placebo) (mmol/L)</p>			<p>Authors stated that GlcN sulphate was effectively and safely administered to 2 patients with diabetes</p> <p>Authors concluded that the intervention was successful at improving articular function.</p>	

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Source	Study Objective and Design	GlcN Dose and Duration	Fasting Glucose	Glucose Challenge	Insulin Levels/ Sensitivity	Conclusions and Other Relevant Observations	Study Quality Rating
	of GlcN (400 mg) during 7 days, followed by 500 mg t.i.d. of oral GlcN. Controls received 1 intramuscular injection of piperazine chlorbutanol for 7 days followed by 14 days of oral placebo tablets. Primary endpoints were degree of pain, and various movement indices. Routine laboratory tests were performed before and after treatment.						
<p>Studies in highlighted in grey indicate glucosamine effect GLcN = Glucosamine; t.i.d = three times a day</p> <p>Study quality rating system was adapted from Health Canada (2003), and was based on the following criteria: I = Systematic reviews and meta analysis of randomized controlled trials II = Clinical trials without randomization and/or control groups. III = Descriptive and observational studies (e.g., correlational studies, cohort studies, case-control studies) IV = Peer-reviewed published articles; Conclusions of other reputable regulatory agencies; Previous marketing experience; Expert opinion reports V = Non-peer reviewed studies.</p>							

APPENDIX B

***Curricula Vitae* of Expert Panel Members**