Glucose and Surgical Sepsis: A Study of Underlying Immunologic Mechanisms

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BACKGROUND: Early clinical trials investigating the role of tightly controlled glucose levels showed marked benefit in survival of critically ill patients. However, a recent meta-analysis and large randomized controlled trial have failed to reproduce the benefit, showing instead substantially increased risk of dangerous hypoglycemia. We sought to investigate the effects of varying glucose concentrations on previously tested, prognostically significant, innate immune parameters, to define any potential effects of glucose at the cellular level.

STUDY DESIGN: After formal approval and informed consent, venous blood samples were collected from young healthy volunteers. Up to 11 corresponding (same-subject) samples were incubated at 100, 350, or 600 mg/dL glucose concentrations and analyzed to determine human leukocyte antigen-DR surface receptor expression, cytokine release, phagocytic capacity, and formation of reactive oxygen species. Data are presented as mean ± SEM.

RESULTS: After incubation, the change in human leukocyte antigen-DR mean channel fluorescence from resting baseline values in lipopolysaccharide-stimulated monocytes was not significantly different between 100, 350, and 600 mg/dL (1,749 ± 110; 1,748 ± 120; and 1,725 ± 96, respectively; p = 0.89). Tumor necrosis factor-α concentrations were significantly lower for samples incubated at higher glucose concentrations (179 ± 50 pg/mL, 125 ± 30 pg/mL, and 107 ± 29 pg/mL; p < 0.05). The phagocytic capacity of the innate immune system was marginally enhanced by glucose. However, the formation of reactive oxygen species was markedly impaired by rising glucose (55% to 66% impairment; p < 0.05).

CONCLUSIONS: Increasing glucose concentrations exert considerable opposing effects on several well-established innate immunologic processes. The opposing findings might contribute to recent clinical controversies. Physician judgment and experience are essential to imminent treatment of critically ill and perioperative surgical patients. (J Am Coll Surg 2010;210:966–974. © 2010 by the American College of Surgeons)

In recent years, attempts to understand innate host defense mechanisms have provided a specific focus for the reduction of surgical site infection and associated sepsis, which extends beyond prophylactic or therapeutic antimicrobial therapy. Manipulating or even failing to control factors such as temperature, oxygen, and glucose, among others, has been shown to substantially influence development of surgical site infection and sepsis after surgery, despite appropriate use of prophylactic antibiotics.

The importance of more tightly controlled glucose levels was once assumed to be clear-cut at both clinical and cellular levels. In reality, that conclusion appears to be radically changing. The early, large, randomized controlled trial by Van den Berghe and colleagues in 2001 rapidly provided the clinical standard of practice, having shown that tight glucose control reduced deaths considerably in critically ill patients. Contemporary acceptance in hospitals led to development of closely adhered to protocols, which were aimed at tightly controlling glucose levels through use of intensive insulin regimens. The practice was extrapolated and expanded to include progressively larger patient populations, including surgical patients. However, in 2008, a meta-analysis that combined all randomized controlled trials investigating the effect of tight glucose control in critically ill medical and surgical patients failed
to reproduce the distinct benefit on mortality described previously. In addition, the meta-analysis highlighted a substantially increased risk of hypoglycemia, with an associated increase in mortality secondary to this overtly dangerous complication. The clinical benefit of tight glucose control was suddenly thrown into question. A large prospective analysis published in 2009 further confirmed the detrimental effect of tightly controlling glucose levels, seen, once again, as a substantially increased mortality rate resulting from associated hypoglycemia.

Because of this current difference of opinion at the clinical level, the benefit and risks from tightly controlling glucose are no longer known and represent a clinical conundrum without a solution in sight. We sought to investigate the effect of increasing glucose concentrations on a wide-ranging spectrum, we hoped that any in vitro effects of varying glucose concentrations would be uncovered, if any, from tight glucose control in hospitalized patients. We used glucose concentrations of 100, 350, and 600 mg/dL, which were chosen to represent a wide spectrum that ranges from normal fasting glucose are no longer known and represent a clinical conundrum without a solution in sight. We sought to investigate the effect of increasing glucose concentrations on a well-recognized, previously tested, and prognostically significant innate immune parameters at the cellular level. Although acknowledging the limitations of in vitro research, we hoped that the translation of such bench laboratory findings to the bedside might aid in uncovering potential effects, if any, from tight glucose control in hospitalized patients. We used glucose concentrations of 100, 350, and 600 mg/dL, which were chosen to respectively represent a wide spectrum that ranges from normal fasting levels through poorly controlled diabetic levels, and extend to a grossly elevated ketoacidotic glucose level. By using such a wide-ranging spectrum, we hoped that any in vitro effects of varying glucose concentrations would be unmasked. We specifically investigated the effects of glucose on monocyte presentation through surface human leukocyte antigen (HLA)-DR receptor expression, cytokine production, neutrophil phagocytosis, and formation of reactive oxygen species (ROS) by neutrophils, using a consistent and reproducible whole-blood model.

METHODS
Whole-blood sampling
After approval by the University of Louisville Institutional Review Board and written, informed consent, up to 15 mL venous blood samples were collected from a random selection of 15 healthy (8 male, 7 female), 18- to 42-year-old volunteers. Volunteer body mass indices (calculated as kg/m²) were all within normal limits (range 18 to 25 kg/m²). Exclusion criteria included any history of immunosuppressive disorders, diabetes mellitus, chronic medication, or pregnancy. All volunteers fasted for a minimum of 6 hours before venipuncture and blood glucose levels were determined using a Glucometer Elite (Bayer Corporation). Subjects with fasting blood glucose levels <90 mg/dL or >110 mg/dL were excluded. Sample identities and concentrations were blinded throughout the experimental procedure and data analysis and equal or near equal (if odd-numbered) numbers of male and female volunteers were recruited for all experiments conducted.

Sample preparation
For HLA-DR receptor and cytokine determinations, blood was collected in ethylenediamine tetraacetic acid Vacutainers (Becton-Dickinson and Co.). Samples were supplemented with 200 mM l-glutamine at a concentration of 10 μL/mL whole blood because incubation periods were >1 hour (Sigma Chemical Co.). Aliquots of whole blood (900 μL) were subsequently transferred into 5-mL Falcon polypropylene culture tubes (VWR). A lipopolysaccharide (LPS; Sigma-Aldrich) concentration of 1 ng/mL, as confirmed by our pilot experiments, provided the endotoxin challenge immediately before incubation and was used to emulate inevitable surgical contamination intraoperatively. Samples were supplemented with 100 μL normal saline, 50 μL normal saline, and 50 μL 5% dextrose solution, or 100 μL 5% dextrose solution, to provide 1 mL of 100, 350, and 600 mg/dL glucose solutions, respectively. Glucose levels were rechecked using the glucometer to confirm the accuracy of the solutions prepared.

For phagocytosis assays, venous blood samples were collected in sodium heparin BD Vacutainers (Becton-Dickinson and Co.). LPS (1 ng/mL) was supplemented and 2-mL whole-blood aliquots were preincubated for 15 minutes at the constituted glucose solutions before commencement of quantification of phagocytosis.

For ROS quantification experiments, blood was also collected in sodium heparin BD Vacutainers. Aliquots of whole blood (1 mL) were transferred into 5-mL Falcon polypropylene culture tubes. Optimal phorbol myristate acetate (PMA; Sigma-Aldrich) concentrations of 100 ng/mL were used to provide cellular stimulus before incubation, as determined by previous studies, and by our own time and dose—response pilot experiments. Working solutions of PMA were prepared by adding 5 μL (5 μg) PMA to 0.5 mL PBS. Ten microliters working solution were transferred into Falcon polypropylene culture tubes. Whole-

**Abbreviations and Acronyms**

DHR = dihydrorhodamine 123  
FITC = fluorescein isothiocyanate  
HLA = human leukocyte antigen  
IL-10 = interleukin-10  
LPS = lipopolysaccharide  
MCF = mean channel fluorescence  
PMA = phorbol myristate acetate  
ROS = reactive oxygen species  
TNF-α = tumor necrosis factor-α
blood samples (940 μL) with the appropriate glucose concentrations were then transferred into the polypropylene culture tubes that contained the added PMA to give a 100 ng/mL whole-blood volume.

Part 1: HLA-DR receptor expression

Experimental design
Corresponding LPS-treated samples with glucose levels of 100, 350, and 600 mg/dL were incubated at 21% (room air) oxygen with 5% carbon dioxide, at 37°C. To determine monocyte HLA-DR surface expression, samples were incubated for 2 hours, as determined by early pilot experiments. Aliquots of cultured samples (50 μL) from the HLA-DR assay were stained immediately before incubation (time zero) and after incubation, and were then subsequently analyzed to determine baseline and final monocyte HLA-DR expression, respectively. Throughout incubation, gentle vortex was applied at 45-minute intervals to ensure cellular homogeneity.

Monocyte CD14+/HLA-DR staining and extraction
Whole-blood samples (50 μL) were stained with fluorescein isothiocyanate (FITC)-labeled antihuman CD14+ and phycoerythrin-labeled anti-HLA-DR antibodies (BD Biosciences) to determine monocyte HLA-DR expression. Staining was carried out for 25 minutes in the culture environment to prevent down-/upregulation of HLA-DR before quantitative binding. Appropriately matched isotype controls were used to determine nonspecific binding thresholds. Manufacturer instructions were followed closely.

After staining, red blood cell lysis was carried out for 6 minutes using ice-cold ammonium chloride, potassium bicarbonate, and ethylenediamine tetraacetic acid (Sigma Chemical Co.) solution. Monocytes were pelleted by centrifugation, washed with 1 mL Dulbecco’s PBS (Sigma Chemical Co.), and fixed in 250 μL 1% paraformaldehyde solution (Polyscience Inc.).

Flow cytometric analysis
Monocyte CD14+ and HLA-DR surface expression were analyzed within 4 hours of cell culture using a FACSCalibur flow cytometer (Becton-Dickinson and Co.). A total of 20,000 events were acquired. HLA-DR mean channel fluorescence (MCF) was analyzed in CD14+ monocytes using Cell Quest software (Becton-Dickinson and Co.).

Part 2: Cytokine assays

Experimental design
Two series of cytokine experiments were conducted. The first series of experiments were conducted simultaneously with HLA-DR assay experiments where, after 2-hour incubation and staining for HLA-DR, whole-blood samples were centrifuged at 3,000 rpm for 12 minutes to obtain an acellular supernatant, which was stored at −81°C for subsequent analysis. The second series of experiments was conducted to provide a time-course analysis of anti-inflammatory cytokine release after endotoxin challenge. Samples were incubated for intervals of 30, 60, 120, and 240 minutes. Time intervals were selected to allow characterization of cytokine trends, as determined by early pilot experiments. After incubation, acellular supernatant was obtained and stored as described.

Cytokine determinations
Plasma tumor necrosis factor-α (TNF-α) and interleukin-10 (IL-10) concentrations were quantified using commercially available enzyme-linked immunosorbent assay kits (e-Biosciences). All enzyme-linked immunosorbent assays were carried out in 96-well plates according to manufacturer’s instructions. Samples were assayed in duplicate, with either recombinant human TNF-α, or IL-10, to generate a standard curve. Enzyme activity was measured at a wavelength of 450 nm on a SpectraMax Plus384 spectrophotometer and data were generated using Softmax Pro software (Molecular Devices) and expressed in pg/mL. Lower limits of detection for TNF-α and IL-10 were 4 pg/mL and 2 pg/mL, respectively.

Part 3: Phagocytosis assays

Preparation and opsonization of FITC-labeled Escherichia coli
A stock solution of FITC-labeled E coli (Invitrogen Molecular Probes) was prepared by diluting 5 mg lyophilized FITC-labeled E coli with Hanks Balanced Salt Solution for a final concentration of 1 mg/mL, where 1 mg bacteria consisted of $3 \times 10^8$ particles/mg. The bacteria were sonicated and stored at −81°C until required for use. When required, the labeled organisms were thawed and washed twice with PBS by centrifugation. To opsonize bacteria, a 10% pooled human serum solution was freshly prepared in PBS and was mixed with equal volumes of bacteria for a final opsonin concentration of 5%. The mixture was incubated for 25 minutes at 37°C with 5% carbon dioxide to allow for opsonization to occur. Excess opsonins were removed by washing bacteria twice with PBS and centrifugation, followed by resuspension to the original bacterial volume.

Experimental design
A final volume of 40 μL/mL whole blood (40 μg) of opsonized bacteria was quickly added to the preincubated aliquots. Samples were incubated at 21% (room air) oxygen with 5% carbon dioxide, at 37°C. Sampling times of 2, 6, 12, 20, and 45 minutes were selected, which adequately
characterized the hyperbolic nature of the phagocytic reaction in early pilot experiments and were sufficient to attain >95% of cells contributing to phagocytosis within 45 minutes. At these times, 50-µL samples were extracted with subsequent red cells lysis, white blood cell pellet isolation, and fixation as described previously.

**Flow cytometric analysis**

Before acquisition using a FACSCalibur flow cytometer, extracellular fluorescent bacteria were quenched using 75 µL trypan blue reagent (Invitrogen Molecular Probes). A total of 20,000 events were acquired for each sample. Monocytes and neutrophils were gated according to light scattering properties and the proportion of fluorescent cells was recorded.

**Part 4: ROS assays**

**Experimental design**

ROS were quantified using dihydrorhodamine 123 (DHR; Invitrogen-Molecular Probes). A 60-µg/mL working solution of DHR was freshly prepared by adding 8 µL stock DHR to 1,312 µL PBS to obtain a final volume of 3 µg/mL whole blood. Immediately after its preparation, 50 µL DHR working solution was added to the combined solution of whole blood, glucose, and PMA. After the addition of DHR, culture tubes were promptly placed within the incubation chambers. Samples were incubated for 30 minutes, as determined by early pilot experiments, at 21% (room air) oxygen with 5% carbon dioxide, at 37°C. After 30 minutes incubation, 50-µL samples were extracted with subsequent red cells lysis, white blood cell pellet isolation, and fixation as described previously.

**Flow cytometric analysis**

Samples were analyzed within 1 hour of cell culture using a FACSCalibur flow cytometer. A total of 10,000 events were acquired. MCF (ROS quantity) was recorded in gated neutrophils using Cell Quest software (Becton-Dickinson and Co.).

**Part 5: Statistical analysis**

Each subject’s blood was incubated across the 3 different glucose concentrations, referred to herein as “corresponding samples” (ie, same-subject). This reduces inter-subject variability and allows use of more specialized tests, such as repeated-measures ANOVA testing. Repeated-measures ANOVA was used to detect significant differences between 100, 350, and 600 mg/dL (3 groups). In the event that a significant difference was detected, multiple-comparison testing to compare all pairwise groups was used to assess whether any pairwise comparisons were significantly different (ie, 100 versus 350 mg/dL, 100 versus 600 mg/dL, and 350 versus 600 mg/dL). Multiple comparisons were carried out using the Holm-Sidak test. The p values presented were adjusted for multiple comparisons.

For each experiment, subjects were recruited from the pool of 15 volunteers, ensuring that no predisposition to either gender had occurred. Data are presented as mean ± SEM. Statistical analyses were performed using Primer of Biostatistics software (version 6.0, McGraw Hill, 2005). Significance was assigned at the 5% level.

**RESULTS**

Whole-blood cells were separated using flow cytometry (Fig. 1). After 2 hours of incubation at the varying glucose concentrations, the change in HLA-DR MCF from resting baseline HLA-DR values at time 0 (provided in arbitrary units) in LPS-stimulated monocytes at 100 mg/dL was 1,749 ± 110. When corresponding samples (same-subject) were incubated at 350 mg/dL and 600 mg/dL, recorded MCF was 1,748 ± 120 and 1,725 ± 96, respectively (p = 0.89) (Fig. 2).

At 2 hours, approximate peak values of TNF-α significantly and progressively decreased with increasing glucose concentrations (Fig. 3A). Compared with 100 mg/dL glucose, where mean TNF-α concentration was 179 ± 50 pg/mL, corresponding samples (same-subject) incubated at 350 mg/dL and 600 mg/dL contained mean TNF-α concentrations of 125 ± 30 pg/mL (p < 0.05) and 107 ± 29 pg/mL (p < 0.05), respectively.

The anti-inflammatory cytokine response, assessed using IL-10 concentrations, demonstrated a similar trend between 100, 350, and 600 mg/dL glucose concentrations (Fig. 3B). All concentrations demonstrated a small early...
rise in IL-10 concentrations, which indicated satisfactory cell viability and function during the entire incubation period. There were no substantial differences in the early anti-inflammatory response between the different glucose concentrations at any point in the first 4 hours.

Figure 4 depicts the hyperbolic response, which is characteristic of neutrophil phagocytosis. Using the selected time periods, approximately all neutrophils had contributed to the process of *E. coli* phagocytosis, indicating an adequate experimental response, technique, and, once again, with near complete cell viability and function. The process is characteristically hyperbolic as early ingestion proceeds at an exponential rate followed by a slower response as neutrophils become saturated with ingested organisms. In the initial exponential phase, 600 mg/dL glucose provided consistently faster rates of phagocytosis, as compared with 350 and 100 mg/dL. Phagocytosis rates, represented by $K_w$, 50 (time for 50% of neutrophils to contribute to phagocytosis) values, were 22% quicker for 600 mg/dL as compared with 350 mg/dL (p < 0.05), and 28% quicker as compared with 100 mg/dL (p < 0.05). Phagocytosis at 350 mg/dL was 8% faster than at 100 mg/dL, although the difference was not statistically significant (p > 0.05).

In order to provide a functional aspect to phagocytosis, ROS formation was measured and compared between the 3 glucose concentrations. Although these values do not specifically represent bacterial killing, they represent the amount of substrate available for the process and have been repeatedly shown to positively correlate with intracellular killing. ROS formation was quantitatively assessed using the arbitrary-valued MCF (Fig. 5). When samples were incubated at 600 mg/dL glucose as compared with 100 mg/dL, there was a statistically significant decrease of 66% in ROS formation (p < 0.05). At 350 mg/dL, there was a 55% decrease compared with 100 mg/dL (p < 0.05). ROS for-
information was not significantly different between 600 mg/dL and 350 mg/dL glucose concentrations (p > 0.05).

DISCUSSION

How tightly glucose levels must be controlled in surgical and critically ill patients is no longer known. Tight control achieved using intensive insulin regimens as compared with conventional regimens was once thought to reduce mortality and septic sequelae in hospitalized patients. The ability to adhere to protocols employing the intensive insulin regimens in time came to represent a marker of the quality of care provided by physicians and hospitals. However, recent prospective trials and meta-analyses, which constitute level I clinical evidence, have failed to reproduce these beneficial outcomes. Using clinical studies that employed tight insulin protocols at blood glucose levels <150 mg/dL in an attempt to maintain levels <150 mg/dL, recent level I evidence has instead shown increased detrimental outcomes, including higher mortality rates as a result of aggressive glucose control. By resorting to in vitro cellular studies, the effects of varying glucose concentrations could be studied on early, previously tested, innate immune parameters, which provide the first line of defense against inevitable contamination after surgery or trauma. These parameters have repeatedly been shown to be of prognostic value in the course of patient recovery after surgery and trauma.

Using an unchanged and reproducible in vitro whole-blood model, our results show several important effects resulting from increasing glucose concentrations. Specifically, increasing glucose concentrations substantially impaired formation of reactive oxygen intermediates available for intracellular killing, but enhanced neutrophil phagocytosis rates and attenuated proinflammatory cytokine release. However, there were no substantial differences in HLA-DR surface receptor expression, despite the extreme range of glucose concentrations used. One would think that such extremes of glycemia would unmask even subtle differences in the host defense process.

ROS formation represents a functional assessment of the efficacy of the early components of the immune system to destroy pathogens immediately after contamination, providing an estimate of patients’ capacity to potentially eliminate early infection before gross dissemination within the host, ie, during the well-described decisive period.11–12 ROS formation might arguably be the most important parameter that we have investigated in this setting, because several anesthetic drugs have been shown to impair the innate immune response and specifically blunt ROS formation. In addition, because the ability of cells to present antigen, produce cytokines, and contribute to phagocytosis ultimately culminate in the process of intracellular killing, this parameter might arguably offset all other neutral findings described at the cellular level. The lethality of diseases such as Chediak-Higashi syndrome, characterized by remarkably effective phagocytosis but ineffective intracellular killing, provides evidence of the importance of effective...
ROS generation. Our results clearly show that increasing glucose concentrations substantially impair formation of ROS and the potential for intracellular killing. This particular cellular finding correlates with early studies favoring tight glucose control to reduce mortality and septic complications in critically ill patients.6

From a technical point of view, we used DHR to detect ROS because it has been shown to detect all available intermediates more sensitively than other stains. It is considered by many to be the most sensitive technique available for accurately quantifying ROS formation and, with it, neutrophil oxidative killing potential.12,13

Impairment of ROS formation was not reproduced among other immune parameters, however. In stark contrast, increasing glucose concentrations were found to substantially attenuate proinflammatory cytokine production. We have previously attempted to explain the clinical significance of cytokine production and benefit derived from attenuation of release of proinflammatory proteins such as TNF-α, an early proinflammatory cytokine released in what seems to be a cascade of mediators. It plays a major role in activation and enhancement of immune mechanisms, but has also been arguably linked to host tissue injury and multiorgan dysfunction from many disparate and disagreeing reports. Some evidence for this pathological role began to emerge in 1985, when pretreatment with antiserum to TNF-α was shown to protect mice against endotoxin lethality in vivo.14,15 Van der Poll and colleagues hypothesized that secretion of proinflammatory cytokines, such as TNF-α, occurred in relatively short-lived and rapid bursts immediately after an insult.16,17 Exaggerated or prolonged early responses, however, were associated with higher morbidity and mortality rates, and were shown to be followed by pathologically increased compensatory levels of anti-inflammatory cytokines, such as IL-10, with a resultant “stunning” of the innate immune processes. Remarkably, IL-10 levels were subsequently shown to serve as a prognostic marker of impending mortality.18 Although our overall view remains that an integrated, sequential, and modulated release of both proinflammatory and, later, anti-inflammatory cytokines are more important than absolute values, the beneficial progressive decrease of TNF-α shown with increasing glucose concentrations was unexpected. This cellular finding coincided with a previously undeclared fatal complication of intensive lowering of glucose levels in the ACCORD (Action to Control Cardiovascular Risk in Diabetes) cardiovascular trial, and resulted in early discontinuation of the study. The authors described the peculiar finding that increased mortality rate was not a result of hypoglycemia in the enrolled patients.19,20 An unfavorable modification of the cytokine profile, as elucidated by our findings, might have accounted, in part, for the heightened mortality, particularly considering the potential detrimental effects of TNF-α levels on cardiac myocyte function.21,22 After all, TNF-α levels are highest at 100 mg/dL glucose and the ACCORD study consisted of type 2 diabetic patients with a propensity for cardiovascular disease as a selection criterion.

Also unexpected was the statistically significant (p < 0.05) dose-dependent enhancement in phagocytosis rates with increasing glucose concentrations. The mere presence of additional substrate (glucose) for this energy-dependent process might have accounted for the rate enhancement of this cellular process. Interestingly, because the enhancement was dose-dependent and persisted at 600 mg/dL, it is likely that the Km of the enzymatic processes that use glucose as an energy source might be able to do so even at these glucose ranges. However, although the small enhancement was shown to be statistically significant, clinical significance of this difference is not known.

Our cytokine and phagocytosis findings clearly oppose ROS results. When translated into clinically meaningful terms, ROS findings are in keeping with early clinical trials, such as the Van den Berghe and colleagues’ trial,4 which support the use of aggressive glucose control to lower glucose serum levels. However, cytokine and phagocytosis findings are more in keeping with the most recent contrary clinical body of evidence. In addition, hyperglycemic effects at the cellular level appear to be comparable past a certain threshold level. Although ROS findings can be argued to be of more functional value and importance, these opposing findings at the cellular level represent the underlying scientific complexity of this debate, which might begin to explain conflicting findings seen at the clinical level, but ultimately contribute to heighten the disagreement.

Differences in early anti-inflammatory cytokine production and HLA-DR surface receptor expression, which have both been repeatedly shown to possess prognostic value,23 surprisingly, failed to demonstrate any differences with increasing glucose concentrations at the early stages of the inflammatory response seen in our model.

Although the specific mechanisms by which varying glucose levels might have accounted for the cellular changes described here have not been elucidated by these experiments, studies are underway to investigate specific pathways that might be enhanced or inhibited by glucose. Of note, however, is that previous studies that attempted to specifically isolate the effects of increasing solution osmolarity failed even to reproduce the cellular findings encountered with increasing glucose concentrations.24 Potential mechanisms to explain the effects of glucose in infection models have included the nuclear factor-κB pathway.24-26
β-O-linkage of N-acetylglucosamine, which is a glucose-related post-translational modification, might also prove important in this context. The intricacy and density of such pathways truly serve to demonstrate the complexity of this topic and the associated findings in the clinical setting.

Although these studies have attempted to shed light on potential processes that might be affected by rising glucose concentrations, limitations related to in vitro experimentation must be kept in mind. These include use of artificial stimulants, such as LPS and PMA; inability to prolong infection models to periods that might allow more complex interactions between various proinflammatory and anti-inflammatory processes to surface, if at all possible; and the understanding that clinical significance of mathematically significant findings might not be truly known outside of the complex interactions that exist in vivo.

In view of the complexity and nature of opposing findings now shown at both the cellular and clinical level, what should treating physicians do to ensure the best outcomes for their patients? We believe that our scientific findings, combined with recent clinical findings, provide an example where rigid protocols do not substitute for expert physician judgment and experience, especially when the available body of evidence suddenly changes. Conventional insulin regimens, which traditionally lower glucose serum concentrations in a moderate, yet timely fashion, largely avoid dangerous complications, such as hypoglycemia, which might not be detected in over-rapid glucose-lowering protocols. Conventional regimens thereby avoid “rollercoaster” glucose levels and provide more controlled maintenance regimens. As a final point, glucose levels should perhaps be adjusted to patients’ individual baseline requirements, rather than to generalized set values for the entire population. Additional clinical and scientific studies suggested here are warranted to help answer one of the most rapidly evolving and debated topics of this century.

Increasing glucose concentrations exert multiple substantial and opposing effects on several well-recognized and previously tested cellular and immunologic parameters. Most importantly, the functional capacity of the innate immune response, reflected by formation of potent killing ROS, is substantially impaired by high glucose concentrations in vitro, but with a paradoxical attenuation of proinflammatory cytokine release and enhancement of phagocytosis. The opposing findings at the cellular level might contribute to the clinical controversies that have thrown recent practice and protocols into disarray. Expert physician judgment and experience are essential in the imminent treatment of one of medicine’s most important recent clinical debates, as additional cellular mechanistic studies and clinical trials continue to emerge.

Author Contributions

Study conception and design: Qadan, Fry, Polk
Acquisition of data: Qadan, Weller, Gardner
Analysis and interpretation of data: Qadan, Maldonado, Fry, Polk
Drafting of manuscript: Qadan, Weller, Gardner
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Acknowledgment: A substantial degree of guidance and supervision were provided by our laboratory manager, the late Mr James D Pietsch, whose contributions included the design and implementation of our in vitro model. We are also grateful to Drs Ozan Akca and Susan Galandiuk for their direction and guidance in the study design process. Finally, we are indebted to the volunteers who donated blood for these studies. Motaz Qadan holds the Joint Royal College of Surgeons of Edinburgh (RCSEd)/James and Emmeline Ferguson Research Fellowship.

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