Resting and exercise-induced IL-6 levels in children with Type 1 diabetes reflect hyperglycemic profiles during the previous 3 days

Jaime S. Rosa,1,2 Rebecca L. Flores,2 Stacy R. Oliver,1,2 Andria M. Pontello,2 Frank P. Zaldivar,2 and Pietro R. Galassetti1,2

1Department of Pharmacology, School of Medicine, University of California, Irvine, Irvine; and 2Institute for Clinical Translational Science, Department of Pediatrics, University of California, Irvine, Orange, California

Submitted 23 September 2009; accepted in final form 8 December 2009

Rosa JS, Flores RL, Oliver SR, Pontello AM, Zaldivar FP, Galassetti PR. Resting and exercise-induced IL-6 levels in children with Type 1 diabetes reflect hyperglycemic profiles during the previous 3 days. J Appl Physiol 108: 334–342, 2010. First published December 10, 2009; doi:10.1152/japplphysiol.01083.2009.—Poor glycemic control in Type 1 diabetes (T1DM) causes long-term cardiovascular complications, at least in part via chronic, low-grade inflammation associated with recurrent hyperglycemia. While physical activity can reduce both inflammation and cardiovascular risks, the underlying molecular mechanisms remain unclear. This is particularly important for T1DM children, for whom the prevention of long-term cardiovascular complications must include optimization of exercise-related anti-inflammatory strategies. We therefore studied the effect of prior hyperglycemia on resting and exercise-induced inflammatory status (plasma IL-6) in T1DM children. Glycemia was continuously recorded with a continuous glucose monitoring system (CGMS) system for 63 h preceding a 30-min intermittent cycling exercise protocol at ~80% peak rate of oxygen uptake (VO2max). Euglycemia (4.4–6.1 mM) was maintained for 90 min before, during, and 30 min after exercise. IL-6 plasma concentration (pg/ml) was measured at baseline, at end exercise, and 30 min postexercise. Subjects were then divided into quartiles based on average glycemia during the CGMS recording. IL-6 levels (pg/ml) were lowest in the quartile with lowest average 3-day glycemia and increased proportionally to greater hyperglycemic exposure; this was observed at baseline (0.86 ± 0.10, 1.06 ± 0.16, 1.14 ± 0.14, 1.20 ± 0.16), absolute IL-6 change (Δ) at end exercise (0.20 ± 0.16, 0.32 ± 0.10, 0.48 ± 0.09, 0.62 ± 0.13), and Δ at 30 min postexercise (0.49 ± 0.13, 0.71 ± 0.16, 0.89 ± 0.14, 1.38 ± 0.33). Therefore, poorly controlled glycemic profile, even in the 63 h preceding an exercise challenge, can alter inflammatory adaptation in T1DM children. Our data underscore the necessity to fully understand all molecular aspects of physical activity to provide the scientific rationale for exercise regimens that will be able to maximize health benefits for T1DM children.

In patients with Type 1 diabetes (T1DM) of all ages, it has been extensively documented that prolonged poor metabolic control has been associated with the development and progression of both microvascular (retinopathy, nephropathy, neuropathy) and macrovascular (coronary artery and cerebrovascular disease) diabetic complications (45–48). While the molecular mechanisms that link chronic hyperglycemia to diabetic vascular complications are still incompletely defined, dysregulation of systemic inflammatory status is now believed to play an important role (13, 50). The etiology of diabetes-related inflammation is complex and invariably includes at least two major components: an “intrinsic” component, i.e., the permanent exaggeration of inflammatory mechanisms related to the presence of diabetes per se (including hyperactivation of specific leukocyte subtypes responsible for the initial autoimmune events leading to disease onset), and a “reversible” component, comprising the inflammatory exacerbations associated with chronic and acute hyperglycemia (43, 50). Except for the few months or years immediately following diabetes onset, the latter component appears quantitatively more important, being related to the frequency and duration of hyperglycemia (15). However, this inflammatory component is also susceptible to correction through strict glycemic control (46, 48). This may be particularly important for T1DM children, in whom increased long-term cardiovascular risk may build gradually over many years starting very early in life, through chronic, subclinical inflammation modulated by hyperglycemic fluctuations (45). Unfortunately, very little information is available, especially in children, on how the characteristics of prior hyperglycemia (severity, duration, repetition pattern) affect subsequent inflammatory status.

Another important factor affecting the balance between glycemic control, inflammation, and cardiovascular risk in T1DM is physical exercise. Exercise training is known to prevent vascular complications in both healthy and T1DM individuals (40); this effect is believed to be mediated, at least in part, by an overall reduction in systemic inflammation (8, 40). T1DM patients, therefore, who are in general at increased risk for vascular diseases, should aim to derive the maximal possible benefits from physical activities. Contrary to the long-term effects of exercise training, however, each exercise bout actually exerts an acute proinflammatory effect, reflected by transient elevations of inflammatory cytokines such as IL-1β, IL-6, and TNF-α (19, 33, 36). Consequently, it appears that a delicate molecular balance must be preserved between acute and chronic inflammatory effects of exercise. If this balance were disrupted, for instance, by exaggerated acute inflammatory responses to each exercise bout, the long-term anti-inflammatory effects may be reduced or lost (8). To date, however, little is known regarding the modulation of inflammatory status during exercise in T1DM children, especially concerning the proinflammatory influence of recent prior hyperglycemia. Our laboratory has recently demonstrated that morning hyperglycemia may intensify secretions of inflammatory mediators during exercise performed in the following few hours (20); the effects of hyperglycemic episodes occurring over the several days preceding an exercise challenge, however, remain unclear.

In the present study, we hypothesized that the severity of hyperglycemia occurring within the previous 3 days for T1DM...
children would correlate with subsequent inflammatory status, both in resting conditions and during exercise. We therefore measured systemic concentrations of the proinflammatory cytokine IL-6 in a cohort of 47 T1DM children, in whom glycemic profiles over the prior 3 days ranged in glycemic control (as documented by continuous glucose monitoring) from excellent to very poor.

**METHODS**

All study procedures were approved by the University of California, Irvine Institutional Review Board (UCI IRB) and performed at the UCI Institute for Clinical Translational Science (ICTS). Participants were children 11–17 yr old, with T1DM diagnosed ≥2 yr before enrollment, no other morbidity, and on no other therapy than insulin administration (via either insulin pump or multiple injections). All participants underwent a preliminary visit, a 3-day continuous glucose monitoring recording, and a main study visit.

**Preliminary Visit**

Subjects and guardians were fully informed of the experimental protocol and risks and signed informed consents and assents. Vital signs, anthropometric measurements, and physical examination were conducted to evaluate health status. Subjects were excluded if they presented with any other acute or chronic disease (infectious illness, asthma, etc.), major or minor injuries, or use of prescribed or over-the-counter medications other than subcutaneous insulin. Thirty-one subjects participated, and 16 returned for a repeat visit; therefore, 47 complete studies were conducted. All subjects responded to a questionnaire regarding their pubertal status to ensure final results were not due to differences in Tanner stage (37).

The children then checked that their blood glucose was between 5.0 and 14.0 mM (90–250 mg/dl) before undergoing a 12- to 14-min preliminary cycling test on a stationary ergometer (Ergoline 800S, SensorMedics, Yorba Linda, CA) supervised by an ICTS exercise physiologist. The work rate was initially set at no resistance and incrementally ramped by 10–20 W/min, or ~10% of predicted maximal workload according to age, sex, and body size (9), until each subject was not able to pedal further due to exhaustion. Peak rate of oxygen uptake (V\(\text{O}_2\)max) and anaerobic threshold (AT), the level of gas exchange at which further pedaling would require anaerobic energy production in the skeletal muscles) were determined breath-by-breath utilizing a standard metabolic cart (SensorMedics, Yorba Linda, CA).

**Continuous Recording of Glucose Before Main Study Day**

Three days before the main study visit, a glucose sensor was inserted into the suprailiac abdominal subcutaneous tissue. The glucose sensor utilizes a glucose-oxidase enzymatic reaction in the interstitial fluid to collect values every 10 s reflective of glucose concentrations; the sensor was connected to a continuous glucose monitoring system (CGMS) device (CGMS System, Medtronic MiniMed, Northridge, CA) that averages these 10-s glucose readings every 5 min. All subjects were instructed to recalibrate the CGMS by inputting their glucometer readings at least 3 times a day, which allows CGMS readings to accurately reflect actual blood glucose concentrations (12). Data recordings utilized for this study started for all subjects at 9 P.M. 3 days before the main study day and continued through glucose normalization procedures on main study visit (see below) (Fig. 1).

**Main Study Visit**

Subjects were asked to awake at 6 A.M. and ingest a light breakfast covering for carbohydrate ingestion according to their regular insulin administration schedule. Subjects on insulin pumps maintained their baseline insulin administration; those on multiple injections were instructed to not inject the long-acting form of insulin after the preceding evening.

All subjects then presented to the UCI ICTS at ~8 A.M., and their vital signs were again recorded. Intravenous catheters were then inserted into the median cubital veins of both arms, one for access to multiple blood draws and the other for infusions of insulin and glucose. Plasma glucose was then checked (Beckman Coulter Glucose II Analyzer, Beckman Coulter, Fullerton, CA), and euglycemia (4.4–6.1 mM, or 80–110 mg/dl) was maintained in this range for the subsequent 90 min, with a baseline rate of intravenous insulin infusion. At the end of this 90-min euglycemic period, exercise was started. If glucose concentrations were initially >6.6 mM (110 mg/dl), euglycemia was established through infusion of intravenous insulin [in addition to the basal rate, 0.9 nmol/h for every 0.6 mM (10 mg/dl) of hyperglycemia gradually tapered down as euglycemic values were approached]. When euglycemia was established, it was then maintained for 90 min, as described above, followed by the exercise challenge. Plasma glucose was checked every 10 min, and small amounts of intravenous glucose were administered as needed if glycemia tended to drop <4.4 mM (80 mg/dl) (Fig. 1).

After the 90-min euglycemic period, participants began the main exercise protocol by pedaling on a stationary cycle ergometer at a workload of half-way between individual AT and V\(\text{O}_2\)max, or ~80% of peak exercise capacity. Subjects exercised 2 min followed by 1 min rest and repeated this sequence 10 times (Fig. 1). As previously demonstrated (14, 42), this protocol activates robust cardiovascular, hormonal, metabolic, and immunological responses and is well tolerated by the pediatric population. During exercise, plasma glucose was checked every 5 min, and euglycemia was preserved with adjustments of intravenous glucose infusion, if needed. Following exercise, subjects rested on an armchair for an additional 30 min, during which euglycemia was also strictly maintained (Fig. 1). In addition to the multiple small blood draws for plasma glucose monitoring, main
blood draws for IL-6, free insulin, and lactate measurements were obtained immediately before exercise, at end exercise, and 30 min after exercise cessation.

**Laboratory Procedures**

Blood samples for IL-6, insulin, and lactate were drawn into sterile B.D. Vacutainer tubes; after centrifugation, plasma was aliquoted and frozen until assay day. On day of assays, all concentrations of plasma IL-6 and free insulin were quantified using high-sensitivity ELISA kits (R&D Systems, Minneapolis, MN and LINCO Research, St. Charles, MO), following polyethylene glycol extraction, respectively. l-Lactate concentrations were determined using the YSI 2300 STAT PLUS Glucose and Lactate Analyzer (YSI, Yellow Springs, OH).

**Statistical Analysis**

Registered data were extracted from the CGMS portable device into Microsoft Windows XP electronic format and processed with the Medtronic MiniMed Solutions Software Version 3.0 (MMT-7310, Medtronic MiniMed, Northridge, CA). To quantify each subject’s glycemic control during the 3 days preceding each study, several variables were calculated: 1) mean glycemia of the entire 3-day recording (9 P.M. of day −3 to 6 A.M. of main study day), or of sections of recording (previous night, i.e., from midnight to 6 A.M. of study day), or days −1, −2, and −3 (12 A.M. to 12 A.M., respectively); 2) total number of hours during the 3-days recording spent with glycemia above 11.1 (200), 13.9 (250), and 16.7 mM (300 mg/dl), respectively; and 3) higher glycemic value on the morning of study day.

Based on each of the above glycemic variables, subjects were divided into quartiles, and respective IL-6 concentrations were analyzed by ANOVA followed, when appropriate, by a Tukey-Kramer HSD post hoc test, a statistical method that adjusts for multiple comparisons, to identify pairwise differences between groups (30). For IL-6 changes during exercise, the mixed model was used to determine any longitudinal change within groups and difference across groups. The Pearson product-moment correlation and linear multiple regression were applied to evaluate the relationship between two numeric variables. Lastly, data for two groups were compared using two-tailed Student t-test, unpaired for independent groups and paired for within the same group preexercise vs. end exercise. Results in tables and figures are presented as group means ± SE, and all data analyses were evaluated at P < 0.05 level of significance using the JMP statistical software (SAS Institute, Cary, NC).

**RESULTS**

**Prior 3-day Glycemia and Subsequent Resting and Exercise-Induced IL-6 Concentrations**

The main study results are presented as comparison of systemic IL-6 concentrations in subgroups of study participants, divided based on glycemic control during the 3 days preceding study day. Mean glycemia during this period varied greatly across participants, with values distributed evenly across a range of 4.3 to 17.7 mM. Based on these 3-days glycemic mean values, subjects were thereafter divided into four quartiles (P < 0.05 across groups): 1) quartile I, 4.3–8.0 mM (mean 6.4 mM); 2) quartile II, 8.0–9.8 mM (mean 9.0 mM); 3) quartile III, 9.8–13.0 mM (mean 11.0 mM); and 4) quartile IV, 13.0–17.7 mM (mean 14.7 mM).

Representative continuous 3-days glucose tracings of an individual from quartile I and from quartile IV are shown in Fig. 2. Progressing from quartile I to IV, the mean number of hours spent in clinically relevant hyperglycemia (>11.1 mM, or 200 mg/dl) also gradually increased (P < 0.05) (Table 1). No difference across quartiles was found in terms of age, sex distribution, Tanner stage, height, body mass index percentile, or V˙O2max (the gold-standard measurement for physical fitness) (Table 1). Also, in all four quartiles, exercise effort on study day 336 HYPERGLYCEMIA, INTERLEUKIN-6, EXERCISE, DIABETES, CHILDREN

*Fig. 2. Representative continuous glucose monitoring system (CGMS) tracings from 2 study subjects: 1 with good glycemic control (top; 3-day average 6.0 mM, no glucose reading above >11.1 mM) and 1 with poor glycemic control (bottom; 3-day average 13.8 mM; 40 h spent >11.1 mM, 31 h >13.9 mM, 19 h >16.7 mM).*

*J Appl Physiol • VOL 108 • FEBRUARY 2010 • www.jap.org*
day was comparable, as documented by similar increases in plasma lactate. There was also no difference across groups in plasma glucose (Table 2) or insulin at preexercise, end exercise, and postexercise.

Baseline plasma IL-6 concentration (pg/ml) was lowest in quartile I (0.86 ± 0.10) and progressively, albeit nonsignificantly, greater in the other quartiles (II: 1.06 ± 0.16; III: 1.14 ± 0.14; IV: 1.20 ± 0.16) (Fig. 3). A similar pattern was also observed at end exercise and 30 min postexercise, with IL-6 values in quartile IV statistically greater than in quartile I (end exercise: 1.06 ± 0.16 vs. 1.83 ± 0.16, P < 0.05; 30 min postexercise: 1.35 ± 0.18 vs. 2.58 ± 0.40, P < 0.05) (Fig. 3). The exercise-induced increase (Δ) in IL-6 over baseline levels was also progressively greater across quartiles: at end exercise the increase was 0.20 ± 0.16 in I, 0.32 ± 0.10 in II, 0.48 ± 0.09 in III, and 0.62 ± 0.13 in IV (P < 0.05, I vs. IV). This pattern was even more pronounced when the increase was measured between baseline and 30 min postexercise: 0.49 ± 0.13 in I, 0.71 ± 0.16 in II, 0.89 ± 0.14 in III, and 1.38 ± 0.33 in IV (P < 0.05, I vs. IV) (Fig. 3).

A similar general pattern of IL-6 levels correlating with magnitude of prior mean hyperglycemia was also maintained if, instead of mean glycemia of the whole prior 3-days period, mean glycemia of each of the prior 3 days, and of the night preceding study day, were separately used as a predictor of study-day IL-6 values (data not shown).

A Different Examination of the Data: IL-6 Levels With Respect to Total Time Spent With Glycemia Above 11.1, 13.9, and 16.7 mM, and to Hyperglycemia Immediately Before the Study

An emerging concept in diabetes control is that, at least concerning inflammatory effects, "not all hyperglycemia are created equal." Similar average glycemia, for instance, can be recorded over several days through different profiles, i.e., with individual readings relatively clustered around the average, or with broad fluctuations about the average, possibly resulting in different inflammatory activation. We therefore also investigated whether additional information could be obtained from the present data by correlating IL-6 profiles, rather than with mean prior glycemia, with exposure to glycemic peaks above set thresholds: 11.1 mM (clinical definition of postprandial hyperglycemia) (1), 13.9 mM (250 mg/dl), or 16.7 mM (300 mg/dl). Finally, we evaluated the additional effect of hyperglycemia occurring immediately before the study on IL-6.

Effect on IL-6 of time spent with glycemia >11.1 mM during prior 3 days. With this analysis, the total pool of subjects was again divided in quartiles, but this time based on the number of hours spent with glycemia above 11.1 mM (quartile I had the fewest hours, quartile IV the most hours); results essentially paralleled those obtained with average 3-days glycemia: baseline plasma IL-6 in quartiles I–IV was 0.86 ± 0.10, 1.06 ± 0.15, 1.14 ± 0.14, and 1.20 ± 0.16, respectively (Fig. 4). Plasma IL-6 levels were significantly different between quartiles I and IV at end exercise (1.06 ± 0.16 vs. 1.78 ± 0.17, P < 0.05) as well as 30 min postexercise (1.37 ± 0.17 vs. 2.54 ± 0.41, P < 0.05) (Fig. 4). Furthermore, the exercise-induced increases in IL-6, from baseline to 30 min postexercise, were also progressively greater in quartiles I–IV (0.51 ± 0.13, q0.71 ± 0.16, 0.91 ± 0.15, and 1.35 ± 0.33, respectively) (P < 0.05, I vs. IV).

When other hyperglycemic cutoffs (13.9 and 16.7 mM) were applied, the same general pattern of higher IL-6 with greater exposure to hyperglycemia was maintained. The dose-response relation between the two variables, however, was not as clear-cut (data from the 2 middle quartiles were practically overlapping) (Fig. 4).

Not surprisingly, therefore, mean 3-days glycemia had the strongest correlation with time spent >11.1 mM (r = 0.969) (Fig. 5) compared with time spent >13.9 or 16.7 mM (r = 0.947 and 0.904, respectively).

Effect on IL-6 of hyperglycemia occurring immediately before the study. Hyperglycemia has been shown to affect inflammatory status in the few hours following its occurrence (20). We therefore attempted to identify whether hyperglycemia occurring on the very morning of study day, just before study procedures, could have a modulatory effect on IL-6 concentrations in addition to mean glycemia during the previous days.

Subjects were therefore divided into four subgroups, based on the single highest glucose reading on the morning of study day. While in general higher glycemia resulted in greater IL-6 profiles, a clear dose-response pattern was not revealed (Fig. 4), but rather a split existed between the combined data of the top two and bottom two quartiles.

### Table 1. Demographic characteristics of quartile groups I–IV by 3-days glycemic control

<table>
<thead>
<tr>
<th>Age, yr</th>
<th>Sex, F/M</th>
<th>Tanner</th>
<th>Height, cm</th>
<th>BMI%</th>
<th>VO2max, ml·min⁻¹·kg⁻¹</th>
<th>*Time &gt;11.1 mM Glucose, h</th>
</tr>
</thead>
<tbody>
<tr>
<td>I (&lt;8.0 mM)</td>
<td>13.5 ± 0.3</td>
<td>47</td>
<td>2.8 ± 0.3</td>
<td>157.4 ± 2.2</td>
<td>66.8 ± 5.2</td>
<td>40.6 ± 1.7</td>
</tr>
<tr>
<td>II (8.0–9.8 mM)</td>
<td>13.7 ± 0.5</td>
<td>8/4</td>
<td>3.5 ± 0.3</td>
<td>159.9 ± 3.4</td>
<td>71.0 ± 6.0</td>
<td>35.9 ± 2.6</td>
</tr>
<tr>
<td>III (9.8–13.0 mM)</td>
<td>14.1 ± 0.2</td>
<td>7/8</td>
<td>3.1 ± 0.2</td>
<td>160.7 ± 8.3</td>
<td>47.9 ± 8.6</td>
<td>39.4 ± 2.5</td>
</tr>
<tr>
<td>IV (&gt;13.0 mM)</td>
<td>13.8 ± 0.5</td>
<td>6/6</td>
<td>3.1 ± 0.4</td>
<td>159.4 ± 4.1</td>
<td>58.5 ± 9.4</td>
<td>38.1 ± 2.9</td>
</tr>
</tbody>
</table>

Data are means ± SE. F, female; M, male; BMI%, body mass index percentile adjusted by age (in months) and sex; VO2max, maximal oxygen uptake; *P < 0.05 between each group.

### Table 2. Plasma glucose concentration (mM) before, during, and after exercise

<table>
<thead>
<tr>
<th>Glucose Level</th>
<th>Preexercise</th>
<th>2nd Bout</th>
<th>4th Bout</th>
<th>6th Bout</th>
<th>8th Bout</th>
<th>End Exercise</th>
<th>15 min Postexercise</th>
<th>30 min Postexercise</th>
</tr>
</thead>
<tbody>
<tr>
<td>(&lt;8.0 mM)</td>
<td>5.2 ± 0.2</td>
<td>5.3 ± 0.2</td>
<td>5.5 ± 0.2</td>
<td>5.5 ± 0.2</td>
<td>5.4 ± 0.2</td>
<td>5.4 ± 0.2</td>
<td>5.4 ± 0.2</td>
<td>5.4 ± 0.2</td>
</tr>
<tr>
<td>(8.0–9.8 mM)</td>
<td>5.6 ± 0.2</td>
<td>5.5 ± 0.2</td>
<td>5.4 ± 0.2</td>
<td>5.2 ± 0.1</td>
<td>5.2 ± 0.1</td>
<td>5.2 ± 0.1</td>
<td>5.2 ± 0.1</td>
<td>5.2 ± 0.1</td>
</tr>
<tr>
<td>(9.8–13.0 mM)</td>
<td>5.9 ± 0.2</td>
<td>5.5 ± 0.2</td>
<td>5.2 ± 0.2</td>
<td>5.2 ± 0.2</td>
<td>5.0 ± 0.2</td>
<td>5.0 ± 0.2</td>
<td>5.0 ± 0.2</td>
<td>5.0 ± 0.2</td>
</tr>
<tr>
<td>(&gt;13.0 mM)</td>
<td>5.3 ± 0.2</td>
<td>5.6 ± 0.2</td>
<td>5.4 ± 0.1</td>
<td>5.4 ± 0.2</td>
<td>5.2 ± 0.2</td>
<td>5.2 ± 0.2</td>
<td>5.2 ± 0.2</td>
<td>5.2 ± 0.2</td>
</tr>
</tbody>
</table>

Data are means ± SE. Groups (I, II, III, IV) are based on quartiles of 3-days glycemic control. There was no difference between groups.
If, however, children in the top two quartiles with the greatest morning hyperglycemia (which showed similar mean IL-6 responses) were pooled together, and then divided again based on mean glycemia over the prior 3 days (Fig. 6), higher IL-6 levels were systematically displayed by children with the worst 3-days glycemic control (baseline: $1.25 \pm 0.11$ vs. $1.12 \pm 0.17$; end exercise: $1.86 \pm 0.12$ vs. $1.56 \pm 0.21$; 30 min postexercise: $2.50 \pm 0.26$ vs. $1.77 \pm 0.23$, $P < 0.05$; and increase from preexercise to 30 min postexercise: $1.25 \pm 0.21$ vs. $0.65 \pm 0.20$, $P < 0.05$) (Fig. 6).

When multiple regression analysis (stepwise backward selection) was performed for all subjects, indeed, high average 3-days glycemia was a significant predictor for elevated IL-6 at 30 min postexercise ($r = 0.520$, $P < 0.001$) and for IL-6 increment from pre- to 30 min postexercise ($r = 0.500$, $P < 0.001$), independent of morning glycemia, demographics, and anthropometrics.

DISCUSSION

This study revealed that in a cohort of Type 1 diabetic children, hyperglycemia of increasing magnitude during the previous 3 days was followed by a proportionally higher inflammatory status (as represented by IL-6) despite subsequent euglycemic rest and exercise. While this general pattern was maintained regardless of how prior hyperglycemia was...
quantified (3-days average, time spent above specific thresholds), it appeared that an additive, proinflammatory effect existed between the most recent hyperglycemic episode and the level of hyperglycemia during the previous days.

Most existing literature in this field have focused on IL-6 concentrations not following, but during hyperglycemia (15, 44). In vitro studies have shown that cultured cells exposed to hyperglycemia will increase their IL-6 secretion (4, 32); in vivo, elevated systemic IL-6 concentrations have been documented in animal diabetic models and for a variety of human conditions (spontaneous and glucose-infused hyperglycemia in healthy, obese, Type 1 and Type 2 diabetic subjects) (3, 11, 23, 49). Along this concept, our laboratory has also recently demonstrated greater systemic IL-6 at baseline and following exercise in T1DM children compared with healthy controls (20). In the present study, however, both resting and exercise measurements were performed after euglycemia had been maintained for at least 90 min, allowing time for washout of previously elevated inflammatory mediators. Therefore, to our knowledge, this is the first report of the effects of prior spontaneous hyperglycemia on subsequent inflammatory status, both in resting and exercise conditions, after prolonged euglycemia had been reestablished.

IL-6, classically considered a cytokine with predominantly proinflammatory effects, is secreted by macrophages and T-lymphocytes following tissue damage and/or microbial infections (27). Its acute secretion in response to harmful stimuli is therefore physiological and stimulates onset of fever, mobilization of acute-phase reactants (such as C-reactive protein), and optimization of leukocyte activity against pathogens and in the healing of physical injuries (27, 35). However, chronically elevated systemic IL-6 (as may be present in diabetes or hyperlipidemia) has been associated with higher risk of cardiovascular events and mortality (6, 29). This association is possibly due to the deposition of excessive glucose and oxidized low-density lipoprotein (LDL)-cholesterol in the endothelium (4, 25), and to the shunt of glucose into biochemical pathways yielding advanced glycation end-products (AGEs) (4). These events trigger leukocyte infiltration into the vascular basement membrane, emission of highly toxic reactive oxygen species (hydrogen peroxide, hypochloric acid, other oxygen radicals), hydrolyzing proteases, and cytokines (TNF-α; IL-1β, IL-6, IL-8), causing destruction of the vascular wall and reduction of luminal patency (2, 22). When sustained for years, such as during prolonged poor diabetic control, these biochemical processes develop into micro- and macrovascular diabetic complications (26, 46, 48). Exaggerated inflammatory and low anti-inflammatory status have also recently been shown to interfere with the preservation of pancreatic β-cell functions and maintenance of appropriate metabolic control in both Type 1 and Type 2 diabetes (28, 39). These considerations underscore the importance of identifying and preventing altered inflammatory mechanisms, including IL-6 secretion, in at-risk populations (among which T1DM children, in this respect, have been surprisingly understudied) (21).

We chose IL-6 as the our main inflammatory marker in part for its high likelihood to yield clearcut differences across our study groups, as consistent with previously documented responsiveness to exercise stimulation (19, 20, 33). While very little has been published in this field in pediatric populations, various forms of acute exercise have been shown to induce robust, intensity-dependent increases in systemic IL-6 both in animals and humans (7, 16). Exercise-induced leukocytosis and leukocyte activation are likely to contribute to this effect.

Fig. 5. Plot of average 3-day glycemia vs. hours spent with glycemia >11.1 in 47 studies in children with Type 1 diabetes.

Fig. 6. Mean IL-6 concentrations, at rest and after exercise, from 29 studies in children with Type 1 diabetes who had morning hyperglycemia (>11.1 mM) on study day. Children were then subdivided into 2 groups according to their mean glycemia over the previous 3 days. A: •, morning HyG and 3-day glycemia quartiles III–IV; ○, morning HyG and 3-day glycemia quartiles I–II. B: filled bar, morning HyG and 3-day glycemia quartiles III–IV; open bar, morning HyG and 3-day glycemia quartiles I–II. Data are group means ± SE. HyG, hyperglycemia. *P < 0.05 vs. group with lower prior 3-day mean glycemia.
(10), but secretion from other tissues, especially skeletal muscle, is considered quantitatively more important (smaller amounts can also be derived from adipocytes, osteoblasts, astrocytes, and neurons) (24). Greater IL-6 gene expression and protein production are, in fact, well-documented in contracting muscles (24, 34), for which IL-6 may serve as a molecular glucoregulatory signal to the liver, mobilizing supplemental energy sources to sustain muscular work (IL-6 from the skeletal muscle can upregulate hepatic lipolysis, gluconeogenesis, and glycogenolysis) (36). It should be noted that in the past few years, the effects of IL-6 on inflammatory homeostasis have been redefined by some authors as immunomodulatory rather than purely proinflammatory, as IL-6 can also, for instance, induce secretion of anti-inflammatory cytokines (IL-10, IL-1 receptor antagonist) from monocytes and other tissues (36, 38). We believe this concept further supports the importance of the necessity for maintaining appropriate, physiological concentrations of IL-6 at rest and in response to various stimuli, in the context of overall pediatric well-being, growth, and development.

A logical extension of our results is the question of whether mean hyperglycemia over a more prolonged period of time [as reflected, for instance, by hemoglobin A1c (HbA1c)] may also similarly regulate inflammatory status. Interestingly, in our subjects, this was not the case, as children with HbA1c above and below 8.0% had similar IL-6 exercise responses (end exercise increase: 0.45 ± 0.06 vs. 0.44 ± 0.13; 30-min postexercise increase: 0.99 ± 0.79 vs. 0.79 ± 0.24). This does not mean that higher HbA1c may not reflect higher chronic inflammation; in fact, the repeated hyperglycemic periods necessarily associated with higher HbA1c likely induced proportional inflammatory exacerbations. These, however, may have resolved if glycemic control was improved in recent days, suggesting that in our subjects, the major contributing factor to inflammatory status, at any given moment, was the duration and depth of hyperglycemia over the previous few days. In this context, same-day, early-morning hyperglycemia deserves particular attention, as we have previously shown that morning hyperglycemia correlates with resting and exercise-induced IL-6 later in the day (20). In the present study, we added the notion that repeated hyperglycemic episodes, if occurring within a short time (hours to 2–3 days), may have an additive proinflammatory effect. Children with morning hyperglycemia, in fact, had greater IL-6 response compared with those with morning euglycemia; among those with morning hyperglycemia, IL-6 responses were greatest if additional hyperglycemia had occurred over the prior few days. In some sense, this appears similar to the “vicious cycle” caused by recurrent hypoglycemia: an episode of hypoglycemia causes blunting of counterregulatory responses to subsequent hypoglycemia, rendering further hypoglycemia more likely to occur, and perpetuating the cycle (17, 18). It is possible that in a similar fashion, a prior episode of hyperglycemia triggers a sustained inflammatory response which, during a subsequent hyperglycemic episode of similar magnitude, causes an even greater inflammatory response, i.e., requiring a longer time to be resolved. This increases the probability that the next hyperglycemic episode will occur before inflammatory status has returned to baseline, thereby keeping diabetics constantly exposed to some level of exaggerated inflammation, unless hyperglycemia is systematically avoided for a prolonged period of time, i.e., several days in succession. This scenario is corroborated by recent intriguing findings by the Bownlee laboratory, who reported that transient hyperglycemic spikes can cause epigenetic and gene expression changes in aortic endothelial cells, resulting in activation of the proinflammatory NF-κB pathway that persisted long after hyperglycemia had been resolved (5, 13); this was paralleled by observation from our laboratory in children with T1DM, in whom hyperglycemia-induced elevations of IL-1α, IL-4, and IL-6 were not corrected after euglycemia had been restored for at least 2 h (41).

Regardless of the method used to assess prior 3-days hyperglycemia, children in the highest hyperglycemic quartile systematically displayed the greatest IL-6 resting and exercise-induced concentrations. In addition to mean 3-days glycemia, however, the duration of glycemia above 11.1 mM was the variable showing the clearest dose-response relationship with subsequent IL-6 levels. This was not surprising, as 11.1 mM is the pathophysiological cut-off for postprandial hyperglycemia and the renal threshold for glycosuria, i.e., a concentration to which the human body is not expected to be physiologically exposed (1, 31). Counterintuitively, however, duration of exposure to even higher hyperglycemia did not appear to further worsen inflammatory status. In practical terms, these data suggest that optimal cardiovascular protection through glycemic control cannot be obtained by eliminating the higher end of daily glycemic fluctuation but must target the systematic avoidance of each, even relatively moderate, hyperglycemic period.

The practical relevance of our observations is apparent when the average glycemic records of our subjects are examined from the clinical point of view. Our subjects population was from, on average, a middle-class environment with supportive families, and adequate access to health care and diabetes support groups; they had a good understanding of the long-term detrimental effects of their disease and were motivated enough to volunteer for our laborious, time-consuming study. Yet most of them could not achieve proper glycemic control: half of the study participants had a mean glycemia of greater than 9.8 mM during the 3-days recording; on study day, morning fasting glycemia was greater than 11.1 mM in 29 of 47 instances (62%), and over 16.7 mM in 10 participants.

In conclusion, our observations define, for the first time, how each glycemic reading may translate into a quantifiable increase of a specific mechanism leading to increased cardiovascular risk in children with Type 1 diabetes during and after exercise. On the one hand, our data reinforce the notion that, in these children, attempting to achieve optimal glycemic control is an absolute necessity. They also suggest, however, that the effective long-term prevention of diabetic vascular complications may require additional interventions, possibly including carefully calibrated exercise regimens and/or, in poorly manageable cases, the use of anti-inflammatory therapy (50). This study was designed to define part of the biochemical and molecular pathways on which such strategies must be based.

ACKNOWLEDGMENTS

The completion of this project was made possible by the work of all University of California-Irvine Institute for Clinical Translational Science (UCI ICTS) staff members. We would particularly like to thank the UCI ICTS biostatistician Dr. Szu-Yun Leu, Ph.D., for guidance on the statistical methodologies.

J Appl Physiol • VOL 108 • FEBRUARY 2010 • www.jap.org
DISCLOSURES

No conflicts of interest were declared by the authors.

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