

Decreased Stimulated Glucose Oxidation and Iodination by Polymorphonuclear Leukocytes from Insulin-Treated Diabetic Subjects

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SUMMARY

To investigate the mechanism of the reported abnormality of polymorphonuclear leukocyte (neutrophil) function in diabetes, the burst of oxidative activity accompanying the killing reaction and the neutrophil iodination reaction were measured in 21 stable, insulin-treated diabetic subjects and 32 nondiabetic subjects of similar age range. Responses to standard killed preparations of *Candida albicans* (*C. albicans*) (glucose oxidation and neutrophil iodination) and *Staphylococcus aureus* (*S. aureus*) (neutrophil iodination) were studied. The iodination reaction was studied using the subjects' neutrophils suspended both in autologous serum and in pooled normal human serum, and the glucose oxidation test was performed using the neutrophils in pooled normal human serum.

There was a significant decrease in the conversion of ^{14}C -1-glucose to $^{14}\text{CO}_2$ in the neutrophils from diabetics [0.47 ± 0.04 (SE) cf. 0.64 ± 0.04 nmol/ 2×10^6 neutrophils/60 min, $P < 0.005$] and in neutrophil iodination in response to *S. aureus* in both autologous serum (diabetics 0.089 ± 0.008 cf. 0.114 ± 0.008 nmol iodine/ 10^6 neutrophils/90 min, $P < 0.025$) and in pooled human serum (diabetics 0.086 ± 0.009 cf. 0.105 ± 0.006 , $P < 0.05$). There was no significant difference in neutrophil iodination in response to *C. albicans*. There was a significant negative correlation between the diabetics' fasting plasma glucose level and neutrophil iodination in response to *S. aureus* in pooled human serum ($r = -0.54$, $P < 0.05$), but not between fasting plasma glucose and glucose oxidation.

It is concluded that there are significant decreases in stimulated glucose oxidation and in the neutrophil iodination reaction (reflecting decreased function of the myeloperoxidase-hydrogen peroxide-halide microbicidal system) in neutrophils from insulin-treated dia-

abetic subjects. These abnormalities may contribute to the defective bactericidal activity of diabetic neutrophils. **DIABETES** 30:256-260, March 1981.

It has long been recognized that diabetic subjects have an increased liability to severe infection. Neutrophil polymorphonuclear leukocytes (hereafter referred to as neutrophils) provide the first cellular defence against invading organisms, and several investigations of the function of neutrophils from diabetic subjects have shown defects in one or more of the steps involved in neutrophil function. In particular, defects in chemotaxis,¹⁻³ adherence,⁴ and phagocytosis⁵⁻⁹ have been demonstrated. The most recent of these reports dissociated the processes of engulfment and bacterial killing, and found that the killing reaction as well as the engulfment process was abnormal in neutrophils from diabetic subjects.⁹ The cause of this defect is unknown.

The steps involved in the intracellular killing process by neutrophils are complex, and include a burst of glucose oxidative activity following engulfment,¹⁰ which is required for optimal bacterial killing,¹¹ activity of the myeloperoxidase-hydrogen peroxide-halide system,¹² as well as several other oxygen-dependent and oxygen-independent steps. In an attempt to clarify the mechanism of the impaired neutrophil killing process in diabetes, stimulated glucose oxidation and the neutrophil iodination reaction as a marker of the myeloperoxidase-hydrogen peroxide-halide system were studied in a group of stable, insulin-treated diabetic subjects. To determine the contribution of abnormalities in the neutrophils themselves, and effects secondary to defective opsonization, the neutrophil iodination reaction was performed both in autologous serum (AS) and in pooled normal human serum (PHS).

MATERIALS AND METHODS

Subjects studied. A group of 21 ambulant, nonobese (less than 115% ideal body weight) insulin-treated diabetic subjects (age range 14-74 yr) (mean 41) was studied after an

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Received for publication 29 May 1980 and in revised form 28 October 1980.

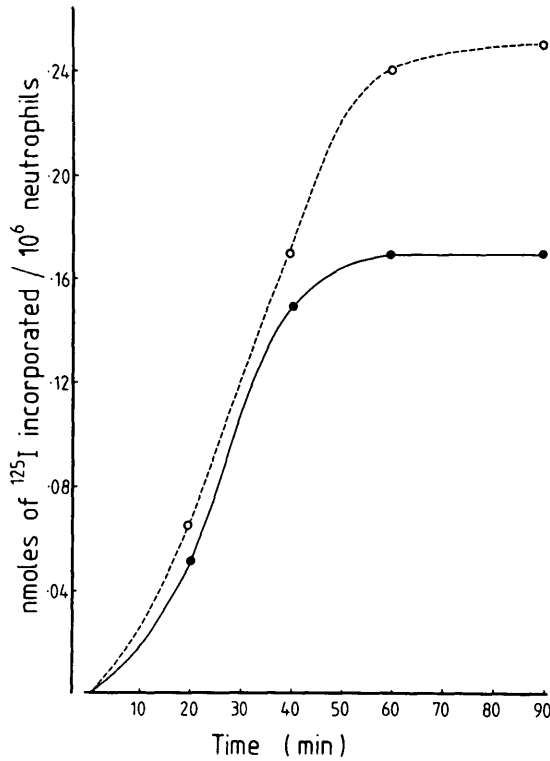


FIGURE 2. Time course of the neutrophil iodination reaction in neutrophils from normal subjects in response to killed preparations (O.D. 1.0) of *S. aureus* ●—● and *C. albicans* ○—○. Each point represents the mean of duplicate determinations.

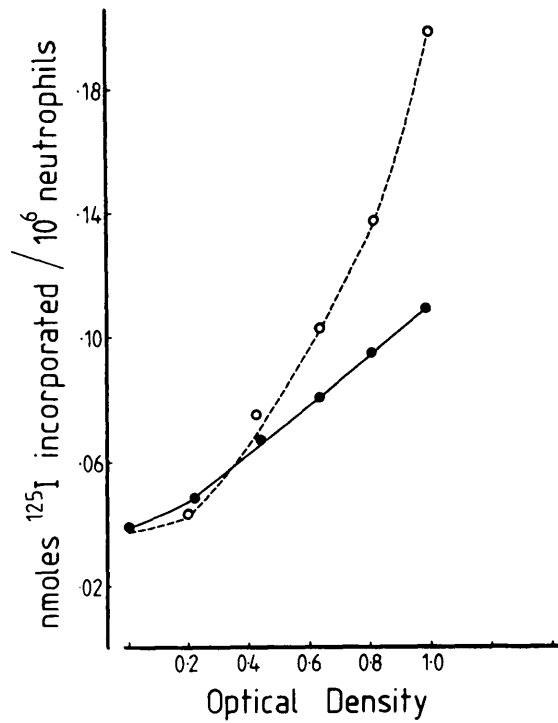


FIGURE 3. Relationship between the concentration of the microbial preparation and the neutrophil iodination reaction. Symbols as in Figure 2. Optical density was measured at 540 nm.

response to *C. albicans* (Figure 1). However, nonstimulated glucose oxidation was identical in neutrophils from nondiabetic and diabetic subjects [0.11 ± 0.01 (SE) nmol glucose/ 2×10^6 neutrophils/60 min in both groups]. There was no correlation between ^{14}C -1-glucose oxidation and the fasting plasma glucose at the time of the test (data not shown). **Neutrophil iodination reaction.** The time course of the neutrophil iodination reaction in response to *S. aureus* and *C. albicans* in neutrophils from normal subjects is shown in Figure 2. It can be seen that a plateau is reached by 60 min. The relationship between the optical density of the microbial preparation and the neutrophil iodination in neutrophils from normal subjects is shown in Figure 3. It can be seen to increase approximately linearly between optical densities of 0.2 and 1.0 for both microorganisms, with a steeper gradient for the *C. albicans*. There was no difference in the basal rate of neutrophil iodination. However, neutrophils from the diabetic subjects showed significantly impaired neutrophil

iodination in response to *S. aureus* in both AS and PHS (Figure 4). There was no significant difference in neutrophil iodination in response to *C. albicans* between neutrophils from normal and diabetic subjects in either AS or PHS (Table 1).

There was a significant negative correlation between neutrophil iodination in response to *S. aureus* in PHS and the fasting plasma glucose at the time of the test in the 16 diabetic subjects in whom simultaneous readings were available (Figure 5).

FIGURE 4. Neutrophil iodination in neutrophils from normal (N) and diabetic (D) subjects in AS and PHS. Mean and SEM are shown for 32 normal and 21 diabetic subjects.

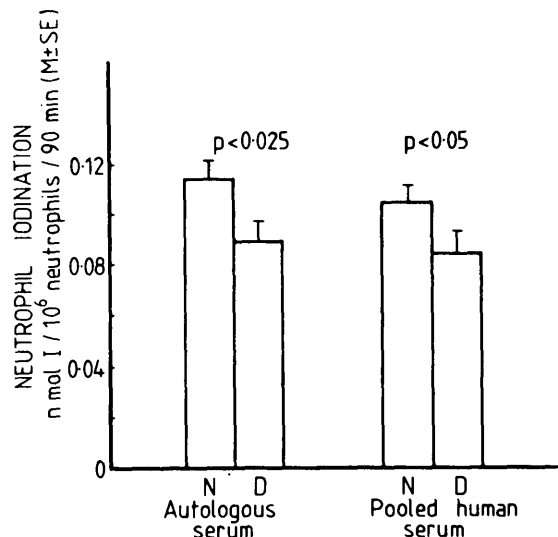


TABLE 1
Neutrophil iodination in response to *C. albicans* in neutrophils from nondiabetic and diabetic subjects in autologous serum or pooled human serum

Source of neutrophils	Source of opsonin	Neutrophil iodination (mean \pm SE) nmol I/10 ⁶ neutrophils/90 min	Signif. cf. control
Nondiabetic	AS	0.23 \pm 0.01	
Diabetic	AS	0.21 \pm 0.01	N.S.
Nondiabetic	PHS	0.23 \pm 0.01	
Diabetic	PHS	0.20 \pm 0.01	N.S.

N.S. P > 0.05.

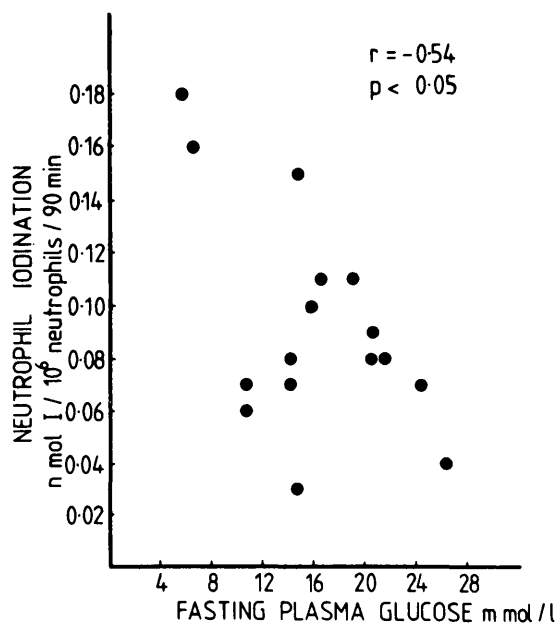


FIGURE 5. Relationship between neutrophil iodination and the fasting plasma glucose at the time of the test in neutrophils from diabetic subjects. PHS was used as the source of the opsonins.

DISCUSSION

This study shows that, in addition to the previously described abnormalities of neutrophil function in diabetic subjects, stimulated glucose oxidation and the neutrophil iodination reaction are also decreased. Before it is assumed that the observed decreases are important primary components of impaired neutrophil function in vivo, other possible explanations must be considered. First, since it has been shown that the engulfment of bacteria by neutrophils from diabetic subjects is impaired,⁵⁻⁹ the possibility that the decreased stimulated glucose oxidation and neutrophil iodination were secondary to impaired engulfment must be examined. Since engulfment is a rapid process, normally complete within 20 min of exposure of neutrophils to bacteria,¹⁸ and since the impaired engulfment observed in diabetic neutrophils at early time phases becomes normal after less than 60 min,⁹ it would appear unlikely that the reduced stimulated glucose oxidation (studied for 60 min) and neutrophil iodination in response to *S. aureus* (studied for 90 min) could be accounted for by the rather modest early decrease in engulfment found in diabetic neutrophils. The time course of the neutrophil iodination reaction, with maximal iodination reached by 60 min (Figure 2), would support this.

In the case of the glucose oxidation experiments, the possibility that the apparent rate of stimulated glucose oxidation depends on the extent of dilution of the specific activity of the added glucose by endogenous glucose produced by glycogenolysis within the neutrophils must be considered. As it is quite conceivable that the rate of glycogenolysis differs in the neutrophils from the diabetic compared with the nondiabetic subjects, this could account for differences in the apparent rates of stimulated glucose oxidation between the two groups of neutrophils. The fact that the basal rate of glucose oxidation did not differ between the groups goes against this possibility, but does not totally exclude it. The neutrophil iodination experiments are not susceptible to this artifact.

The neutrophil iodination reaction measures the capacity of neutrophils to generate H_2O_2 and utilize available halide for microbial killing.¹⁹ Together with the generation of superoxide and hydroxyl radicals, the myeloperoxidase- H_2O_2 -halide system comprises the oxygen-dependent antimicrobial system of the neutrophil, which has a major role in the killing reaction.²⁰ The fact that the reaction was normal with *C. albicans* probably reflected the strong stimulus provided by this preparation at the concentration used (Figure 3), and may demonstrate that the neutrophils from diabetic subjects can react normally if the stimulus is great enough. Alternatively, it may have reflected different mechanisms by which *C. albicans* might activate the myeloperoxidase- H_2O_2 -halide system. These possibilities require further clarification. The decreased stimulated glucose oxidation in the neutrophils from the diabetics shows that the neutrophil response to *C. albicans* is not normal in all respects.

The cause of the decreased neutrophil iodination in the neutrophils from the diabetic subjects is unclear. There is obviously considerable overlap with normal, commensurate with the fact that the subjects were studied when ambulant, stable, and free from infection. A similar overlap was found when the overall effectiveness of bacterial killing was studied.⁹ The negative correlation between neutrophil iodination and fasting plasma glucose suggests that the defect in the neutrophil iodination reaction may depend on the degree of metabolic abnormality. This is compatible with the previous observation that intracellular killing in neutrophils from diabetics improved with improved metabolic control.⁹ The experiments in the PHS showed that the abnormality was associated with the neutrophils themselves rather than with a circulating serum factor. The role of insulin deficiency (relative or absolute) is not clear. Mowat and Baum found that the defect in chemotaxis of the diabetic leukocyte was corrected by incubation of the cells with insulin.¹ Insulin in high concentrations has been shown to have effects on glucose metabolism in diabetic, but not in normal, neutrophils,^{21,22} but no studies have been performed to examine a possible role of insulin in the myeloperoxidase- H_2O_2 -halide system. The demonstration of direct actions of insulin on intracellular H_2O_2 production in adipocytes²³ raises the possibility that insulin may have a direct effect on this system, but this requires further investigation.

In conclusion, the present study has shown abnormalities at two important steps involved in the killing reaction in neutrophils from diabetic subjects. The biologic significance of these abnormalities and further delineation of the pathogenic mechanisms involved in the abnormal function of neutrophils from diabetics and the consequent increased liability to infection awaits further investigation.

ACKNOWLEDGMENTS

This work was supported by grants from the Department of Veterans' Affairs and the John Claude Kellion Foundation. We thank Dr. Cliff Hosking of the Royal Children's Hospital, Melbourne for technical advice.

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