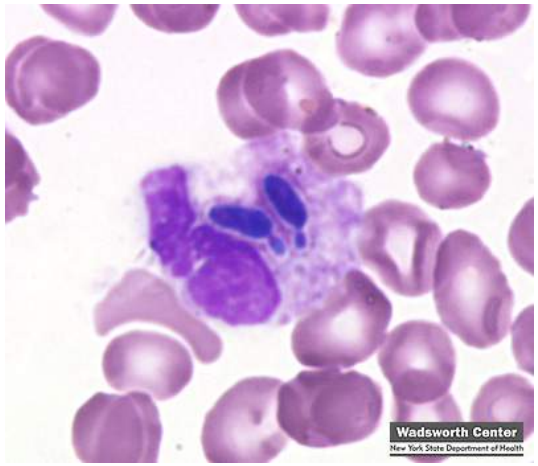
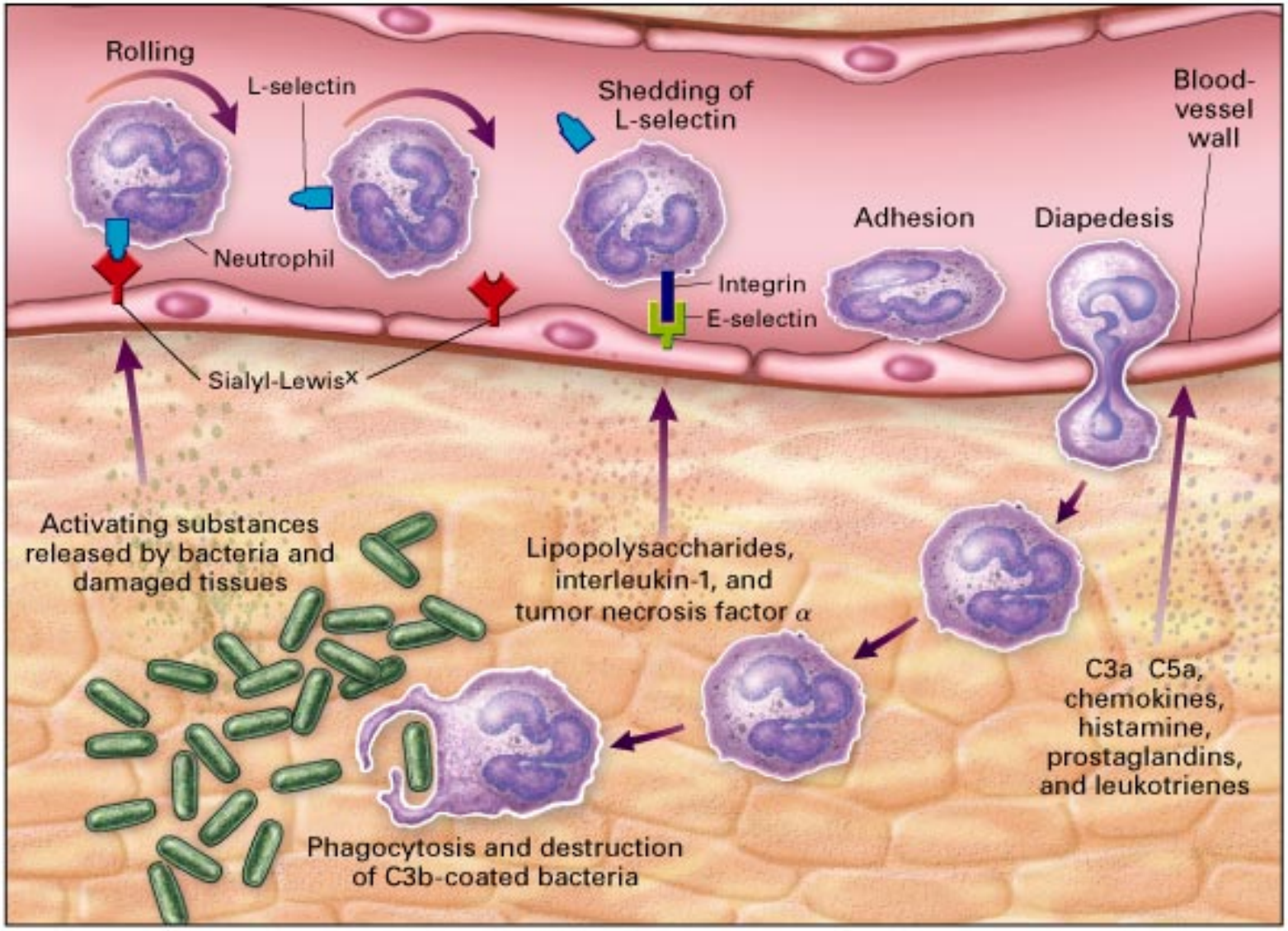
Diabetes Mellitus ve Nötrofil Fonksiyon Bozuklukları

Dr. Oral Öncül
GATA Haydarpaşa Eğitim Hastanesi
Enf. Hst. ve Kl. Mik. Srv.

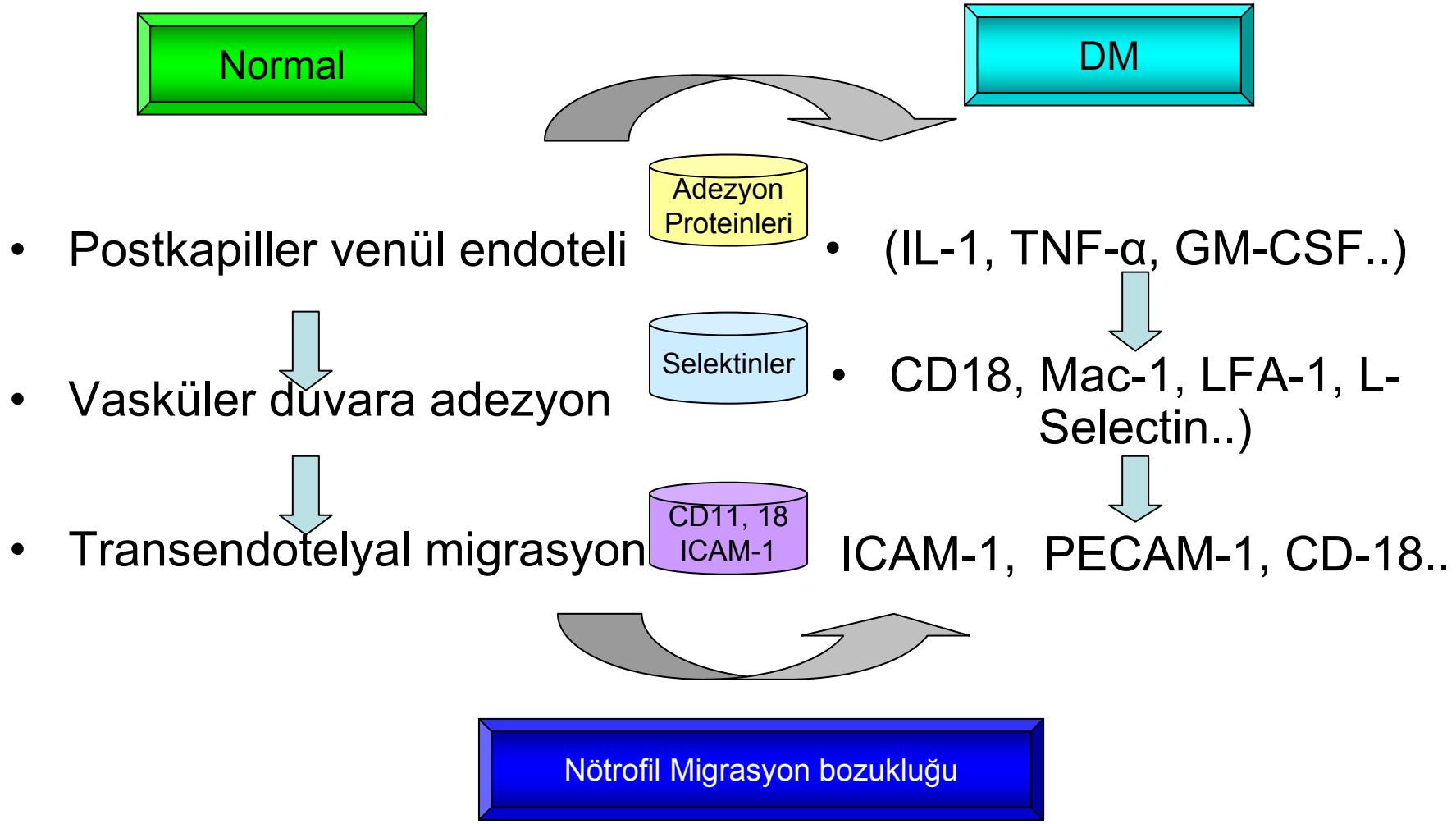
Nötrofil



- Bakterilere karşı ilk hücresel mekanizma
- Akut inflamasyonun önemli bir parçası
- İnflamasyon bölgesine migrasyon,
- Vasküler adezyon, penetrasyon
- Opsonizasyon (Polimerik IgA ab)
- Fagositoz
- Lizozimlerin fagozomla birleşmesi (Fc gama RII, tirozin fosforilasyonu)
- Granül salınımı (Fc gama RIIIB)
- Öldürücü etki
  - Solunumsal patlama (Fc gama RIIIB, CR3, Fc gama RII)
  - Nitrik oksit yolu (NOS I, NOS II, NOS III)
  - Solunum dışı öldürücü etki (Matriks ve primer azurofilik granüller..)


Rolling
L-selectin
Shedding of
L-selectin
Blood-
vessel
wall
Adhesion
Diapedesis
Neutrophil
Integrin
E-selectin
Sialyl-Lewis^x
Activating substances
released by bacteria and
damaged tissues
Lipopolysaccharides,
interleukin-1, and
tumor necrosis factor α
C3a C5a,
chemokines,
histamine,
prostaglandins,
and leukotrienes
Phagocytosis and destruction
of C3b-coated bacteria

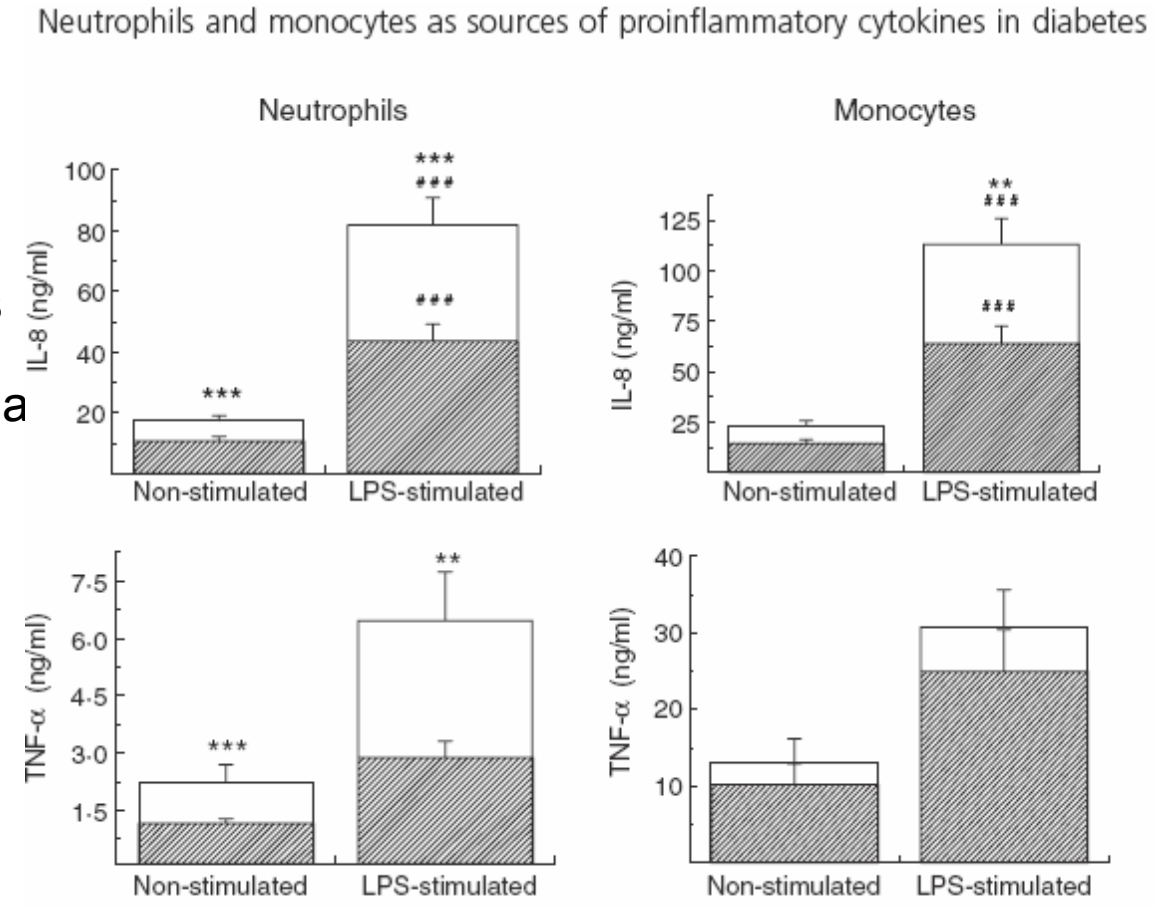
İnflamasyon



Lin JC, et al. J Clin Endocrin Metabol, 2006; 23: 1210

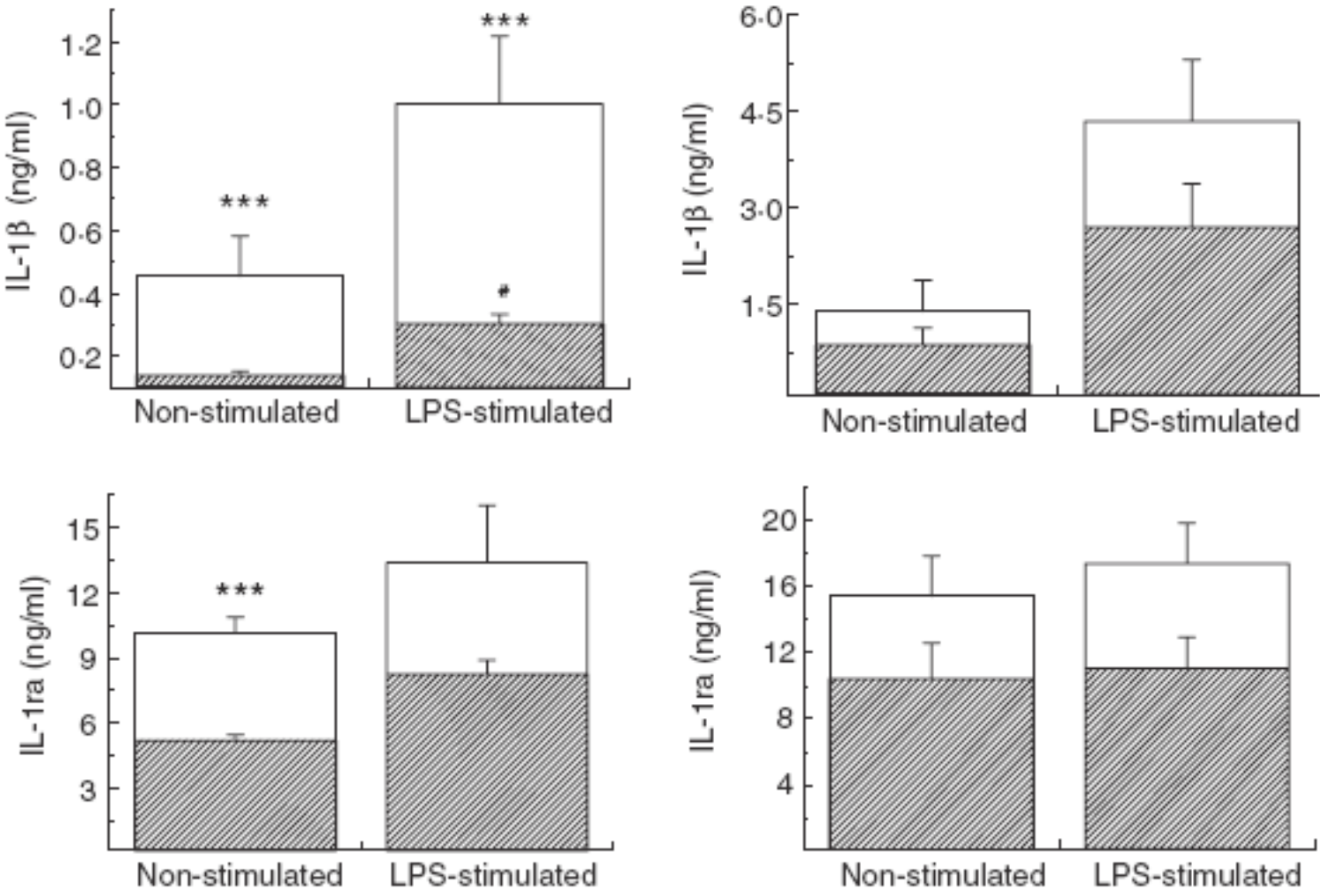
Artan Sitokin Salımı

- İnflamatuar aktivite artışı
- Artmış doku hasarı
- Bakteri duyarlılığında artış
- İlerleyici mortal infeksiyonla



•Hatanaka E, et al. Clin Experimental Immunol, 2006

Artan Sitokin Salımı



•Hatanaka E, et al. Clin Experimental Immunol, 2006

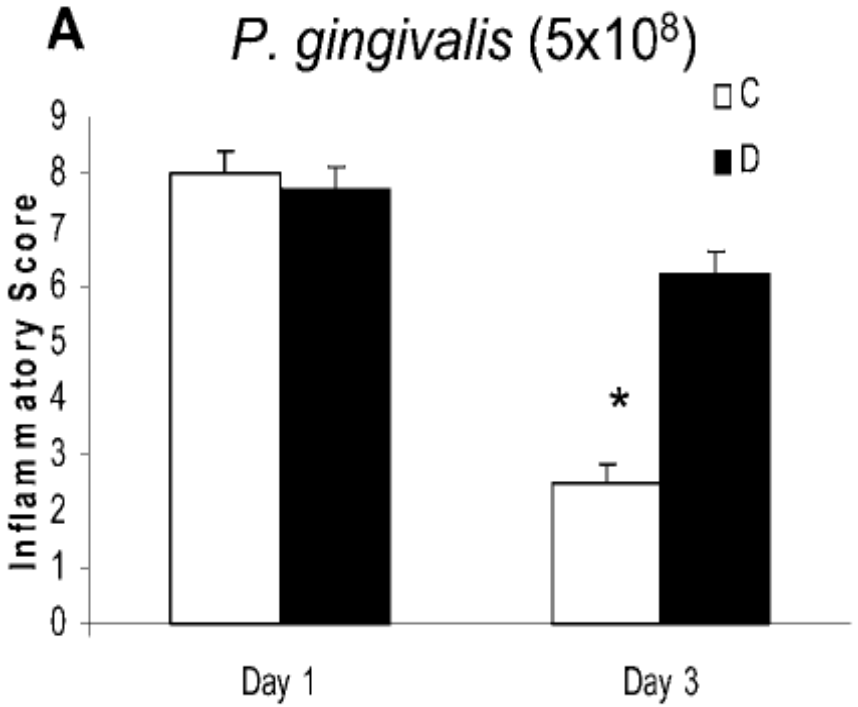
RESEARCH REPORTS
Biological

D.T. Graves*, G. Naguib, H. Lu, C. Leone, H. Hsue, and E. Krall

Department of Periodontology and Oral Biology, Boston University School of Dental Medicine, Suite W-202D, 700 Albany Street, Boston, MA 02118, USA; *corresponding author, dgraves@bu.edu

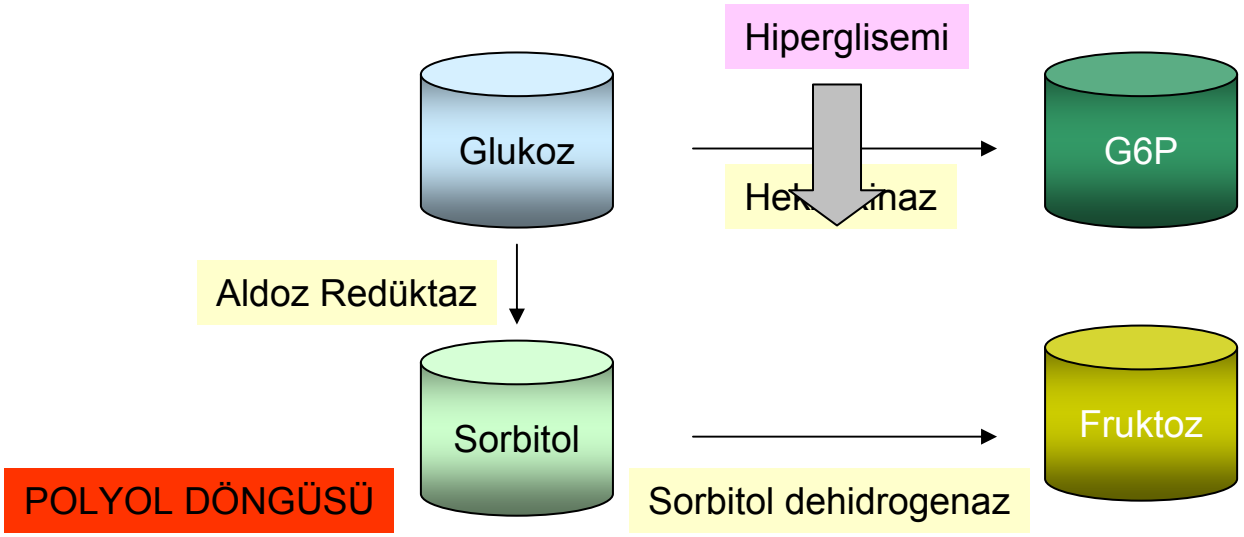
J Dent Res 84(4):324-328, 2005

Inflammation is More Persistent in Type 1 Diabetic Mice



Diabetes Mellitus uzamış inflamatuar etkiye neden olmaktadır.

Lökosit Endotel İlişkisi



- Sorbitol artışı:
  - Hücre içi osmolarite artışı
  - NADPH kullanımının azalması

Lökosit – Endotel İlişkisinde bozulma

Lin JC, et al. J Clin Endocrin Metabol, 2006; 23: 1210

Journal of Leukocyte Biology 42:519–523 (1987)

Evidence of Polymorphonuclear Neutrophils (PMN) Activation in Patients With Insulin-Dependent Diabetes Mellitus

B. Wierusz-Wysocka, H. Wysocki, H. Siekierka, A. Wykretowicz, A. Szczepanik, and R. Klimas
Institute of Internal Medicine, Academy of Medicine, Poznań, Poland

Polymorphonuclear neutrophils' chemotaxis, surface charge, superoxide anions generation, NBT (nitro blue tetrazolium) reduction and intracellular lysozyme, and β-glucuron-

TABLE 1. Chemotaxis of Polymorphonuclear Neutrophils (PMN) Isolated From Patients with Type I Diabetes Mellitus and From Controls Toward Zymosan-Activated Plasma (ZAP), Plasma Incubated With Cellophane (PC), FMLP, and Supernate From E. Coli Culture (Index of chemotaxis)[a]

	Controls (n = 50)	Diabetic patients (n = 30)	Significance of differences
ZAP	2.09 ± 0.15	1.84 ± 0.12	$p < 0.001$
PC	1.76 ± 0.11	1.60 ± 0.14	$p < 0.001$
FMLP	2.11 ± 0.21	2.03 ± 0.13	$p > 0.05$
E. coli	1.87 ± 0.26	1.88 ± 0.17	$p > 0.05$

[a]mean values ± SD.

Kemotaksis ve Degranülasyon

- İndirekt Kompleman Yolunda Bozulma
  - C5 aktivasyonu sonucunda degranülasyon
  - İntravasküler kompleman artışı
  - C kaynaklı kemotaksis fonksiyon bozukluğu
- Direkt Kompleman yolunda bozulma

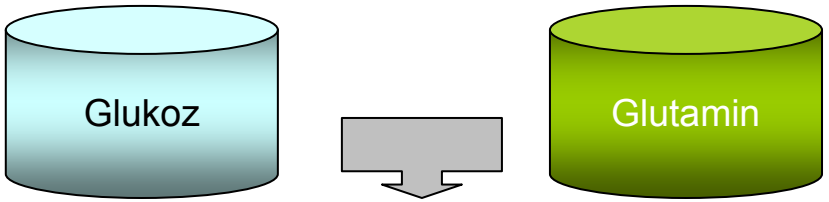
Granül	Azürofil	Spesifik	Jelatinaz	Sekretuar veziküller
Marker Enzimleri	Myeloperoksidaz	Laktoferrin	Jelatinaz	Alkalin fosfataz
Membran	CD63, CD68, Trombospondin..	Sitokrom B, Fibronektin, Laminin..	Sitokrom B, Rap1A, C3bi R..	Sitokrom B, C1qR, Rap1 A..
Matriks	Myeloperoksidaz, Lizozim..	Jelatinaz, Kollejenaz, Histaminaz..	Jelatinaz, Asetiltransferaz..	Plazma proteinleri

Nötrofil Metabolizması

- Tüm fonksiyonlar için enerji gereklidir
- Glukoz ⟹ Laktat (%90-95)
- Krebs siklusu ⟹ Glukoz oksidasyonu (%3)
- Glutamin kullanımı ⟹ (Aspartat, laktat, glutamat, CO2)

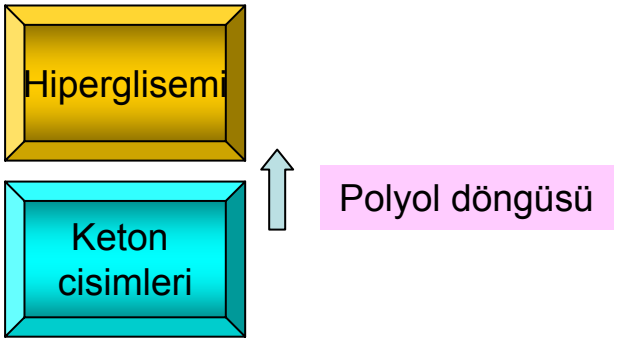
Nötrofil Metabolizması

NORMAL



ATP

DİABETES MELLİTUS



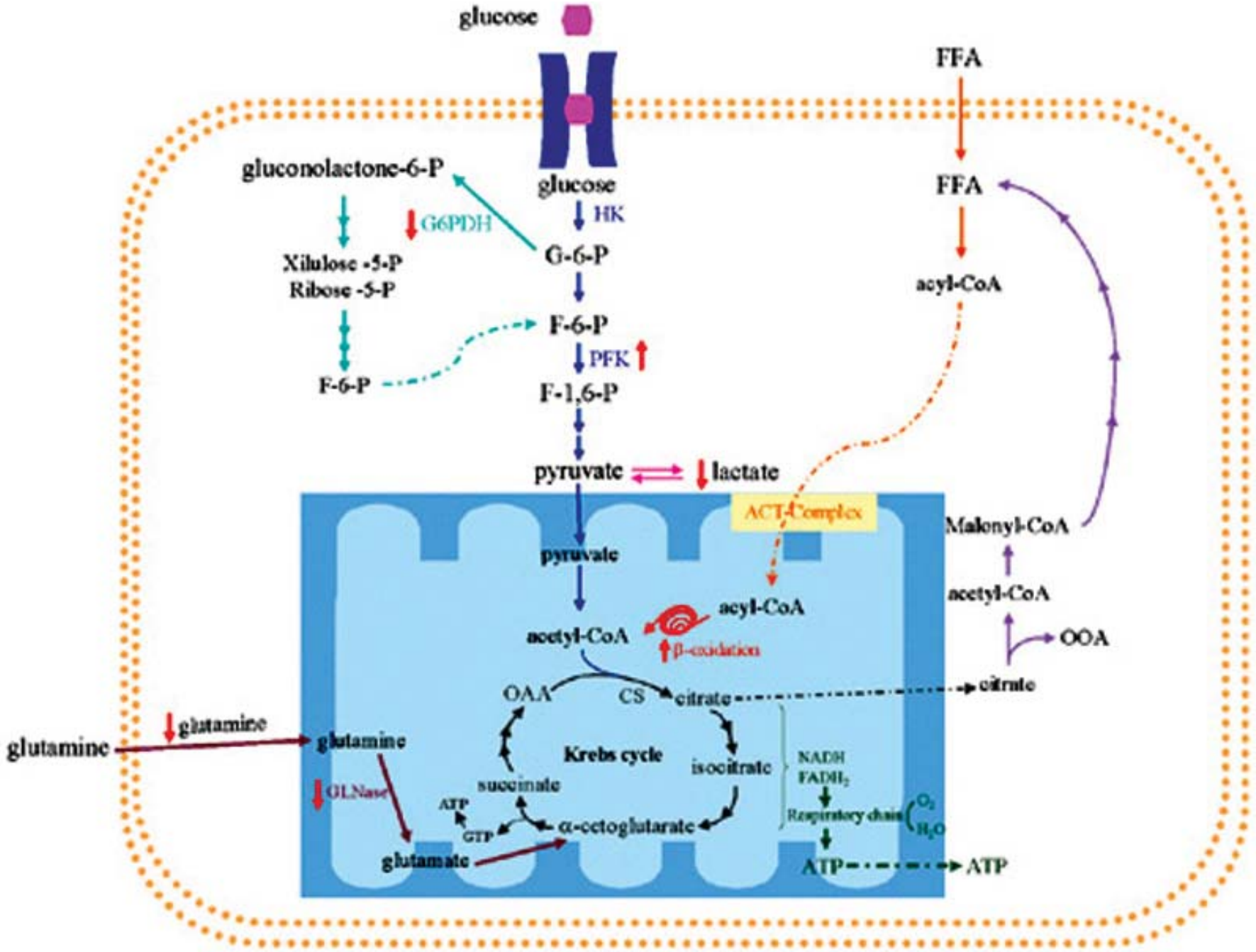
- Glutamin ⇨ Glutamat ⇨ Aspartat ⇨ Laktat ve CO2
- Glukoz ⇨ G6P ⇨ (ATP)
- Metabolitler ⇨ Yağ asidi ve fosfolipid

⇩

Normal Nötrofil Fonksiyonları

- •Laktat üretimi ↘
- •Fosfofruktokinaz üretimi ↗
- •Fruktoz 6 fosfat
- •Fruktoz 2-6 bifosfat

Nötrofil fonksiyon bozukluğu



T.C. Alba-Loureiro Braz J Med Biol Res 2007; 40: 1037-1044

Metabolik aktivite artışı

- NADPH Oksidaz aktivitesinde bozulma
- Süperoksid anyon (O2) düzeyinde azalma
- Respiratory burst (Solunumsal patlama)
- Elektronegatif yüzey geriliminde azalma
- Uyarıya bağlı degranülasyonda azalma

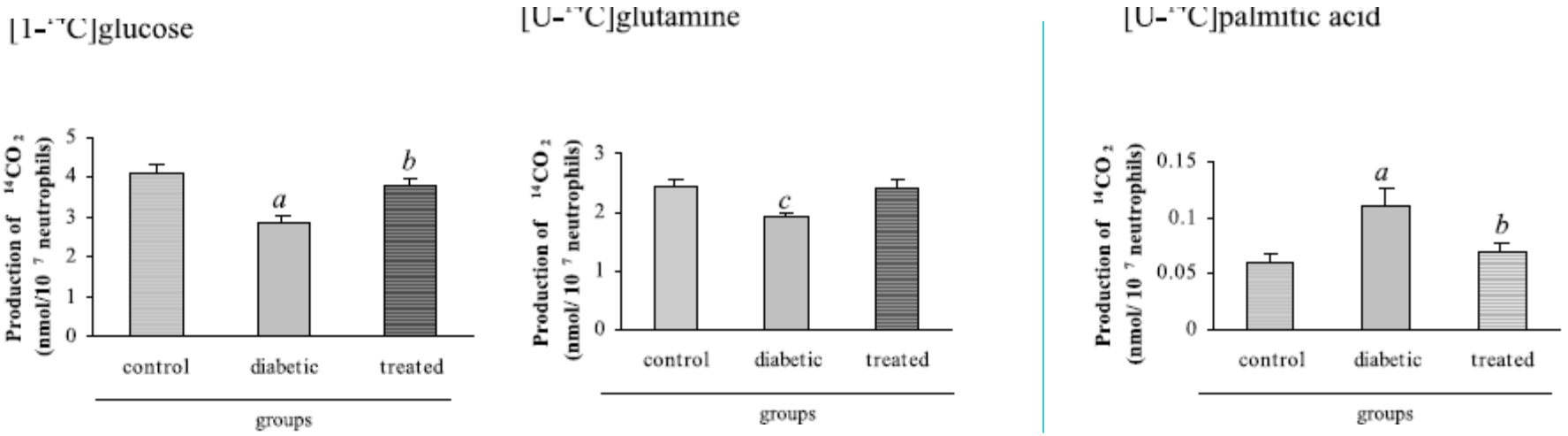
  - Weissmann G. J Lab Clin Med, 1982

Diabetes causes marked changes in function and metabolism of rat neutrophils

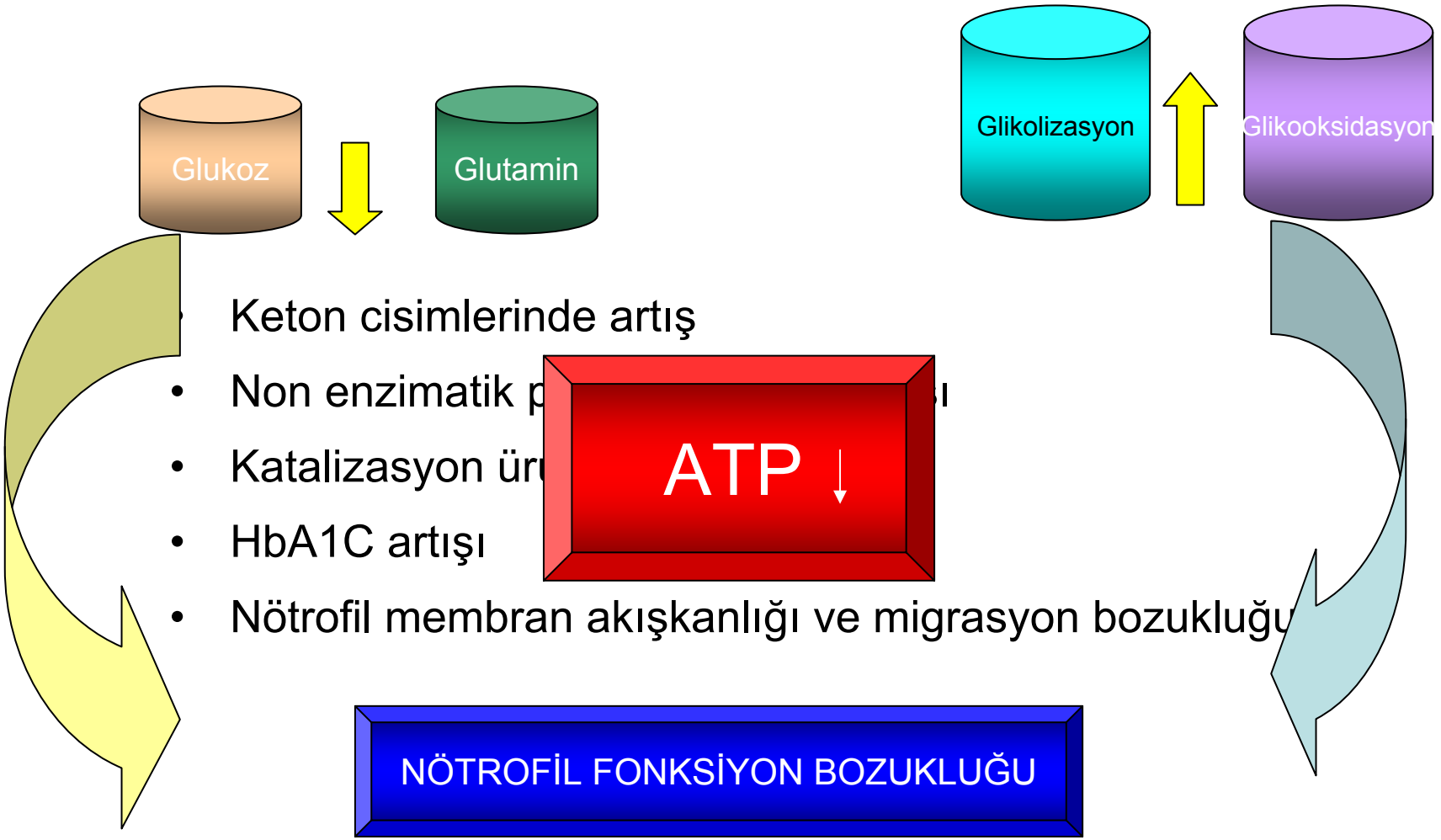
T C Alba-Loureiro, S M Hirabara, J R Mendonça, R Curi and T C Pithon-Curi[1]

Department of Physiology and Biophysics, Institute of Biomedical Sciences, University of São Paulo, Av. Prof. Lineu Prestes 1524, 05508-900 São Paulo SP, Brazil

[1]Institute of Health Sciences, University of São Judas Tadeu, Taquari, 546-Mooca, 03166-000 São Paulo SP, Brazil

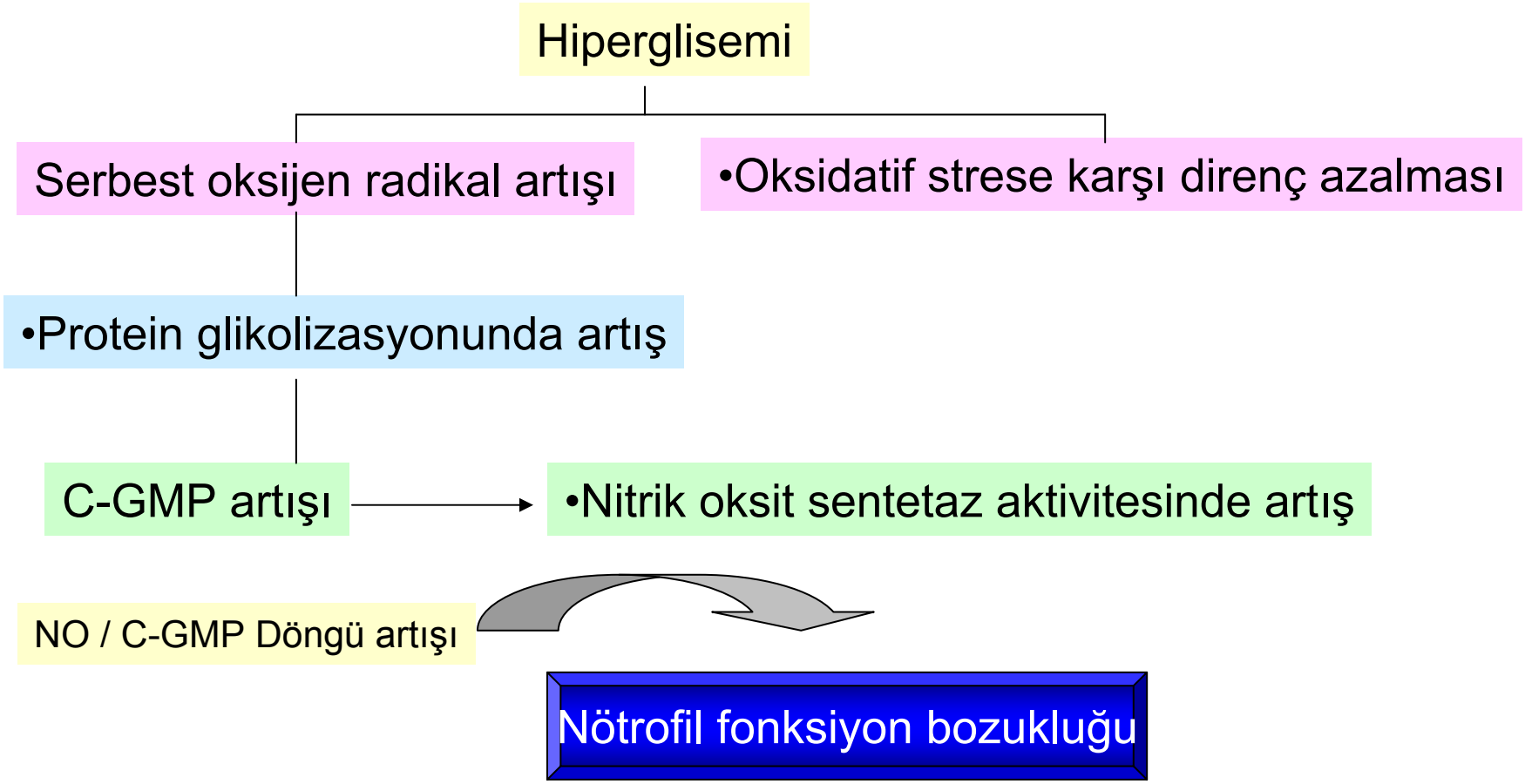


Migrasyon Kapasitesi

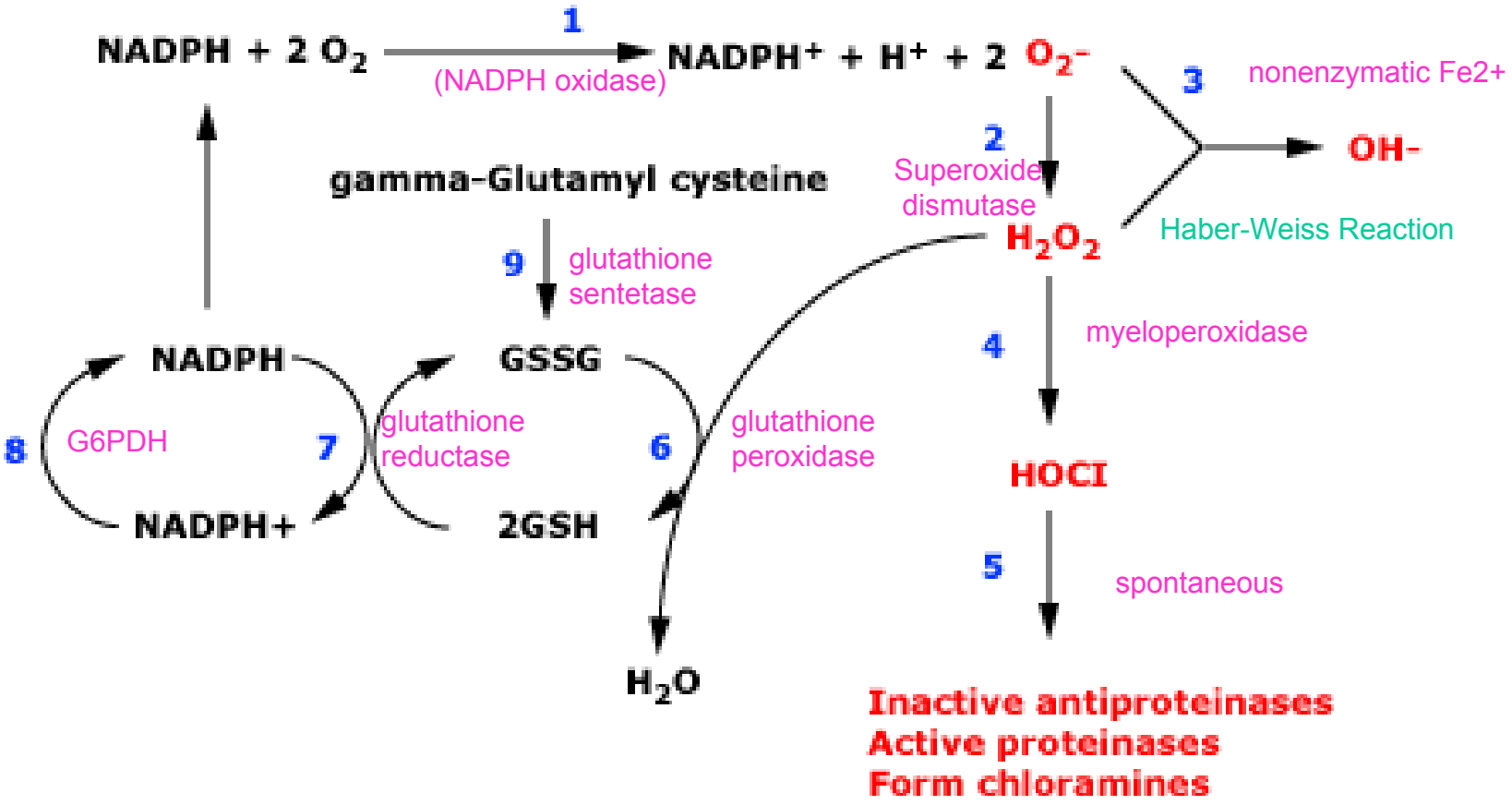


Lin JC, et al. J Clin Endocrin Metabol, 2006; 23: 1210

Nötrofil Serbest Oksijen Radikalleri



Nötrofil Kaynaklı Solunumsal Patlama Döngüsü



Curnutte JT, Orkin SH, Dinauer MC. Genetic disorders of phagocyte function. In: Stamatoyannopoulous G, ed. The molecular basis of blood diseases, 2008

Diabetes Mellitus

- Glukolik ve glutaminolitik yolda bozulmalar
- Nitrik oksit-siklik guanozin 3'-5'monofosfat yolu
- Polyol yolu ile glukoz ve keton cisim artışı
- Glikoliz ve glikojen sentezinde azalma (% 40-60)
- İnsülin eksikliği HMP yolunda aksamalar, düşük antijen yanıtı
- Nötrofil fonksiyonlarında bozulma

Table 2 Consumption and production of metabolites by 1-h incubated neutrophils from the control, diabetic and insulin-treated diabetic groups

	n	Glucose consumption	Lactate production	Glutamine consumption	Glutamate	Production of aspartate	Lactate
Groups							
Control	6	855 ± 170	945 ± 65	1110 ± 95	390 ± 5	515 ± 50	215 ± 5
Diabetic	5	755 ± 150	700 ± 65[a]	1015 ± 70	380 ± 10	555 ± 65	230 ± 40
Insulin-treated diabetic	6	815 ± 150	935 ± 35	1115 ± 85	390 ± 5	510 ± 60	230 ± 35

Consumption of glucose and glutamine and production of lactate, glutamate and aspartate were determined in 1 h incubated neutrophils. The cells were obtained from the peritoneal cavity of the rats, and the results are expressed as nmol/h per mg protein. The values are presented as means ± S.E.M. *n* represents the number of rats per group. [a]$P>0.05$ as compared to control and insulin-treated diabetic rats.

Nötrofil Fonksiyon Bozuklukları

- İnflamasyon
- Endotel adezyonu
- İnflamasyon bölgesine migrasyon
- Opsonik aktivite
- Kemotaksis
- Bakterisidal aktivite
- Fagositoz
- Reaktif oksijen türlerinin üretiminde bozukluk

İnflamasyon

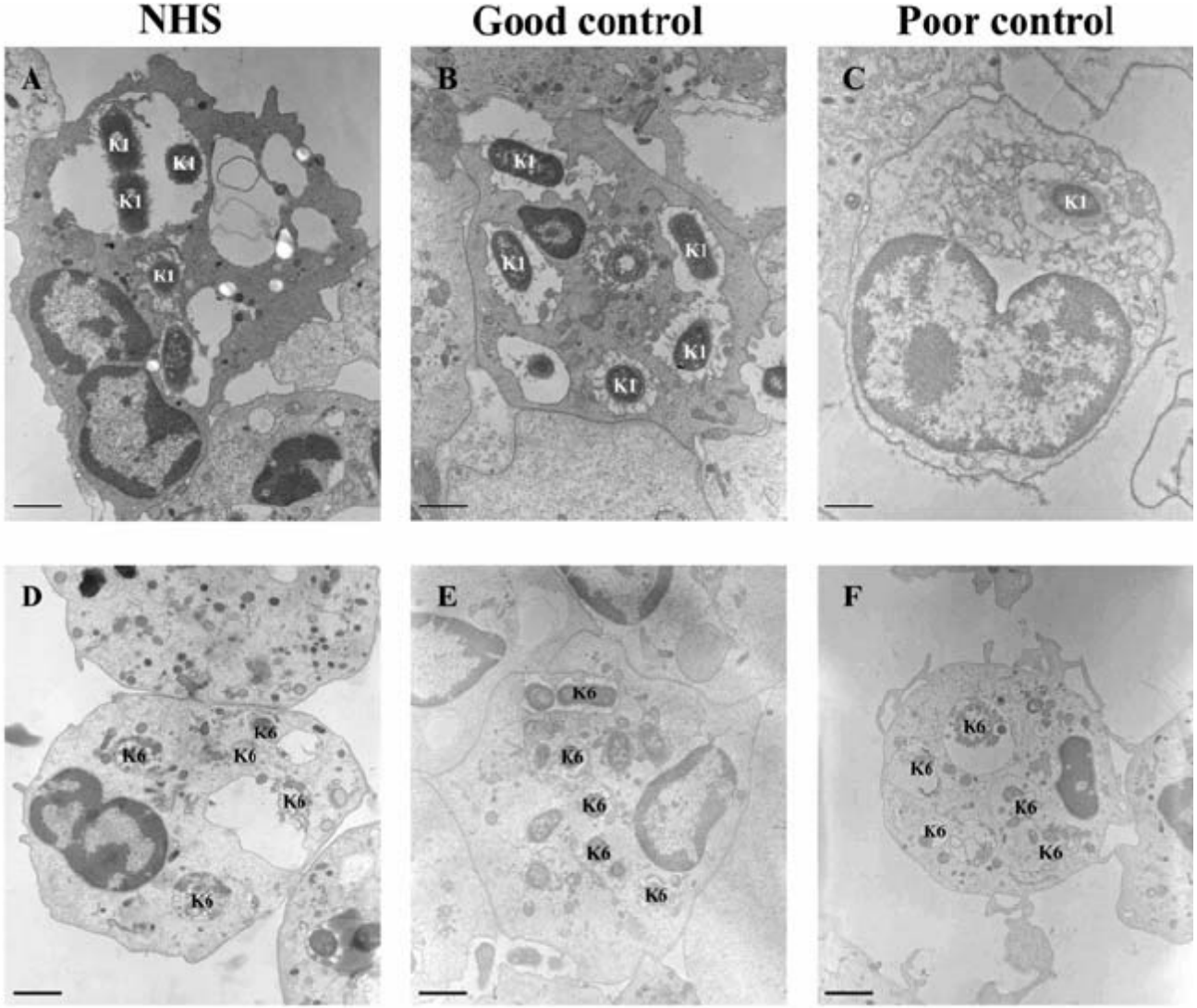
- Azalmış mikrovasküler yanıt
- Protein balans bozukluğu
- Ödem oluşumu ve ödem yanıtında azalma
- Mast hücre degranülasyonunda azalma
- Nötrofil adezyon bozukluğu
- Migrasyon kapasitesinde azalma
- Reaktif oksijen ürünlerinde artış
- Sitokin ve prostoglandin salınımında artış
- Lökosit apopitoz artışı
- Lenf nodu retansiyon kapasitesinde azalma

DM ve Nötrofil Apopitozu

- DM ve Prediabetiklerde LPS'e bağlı erken apopitoz düzeyinde azalma
- Fonksiyon bozukluğu artan nötrofil artışı
- Kemik iliğinde maturasyon etkilenmesi
- Anti-apopitotik sitokin (IL-8, 10) artışı
- Proapopitotik reaktif oksijen ürünlerinde artış
- LPS ile ilişkili IL-12 artışı ve Th1/Th2 (Th1)
- İnflamasyon ve otoimmun DM

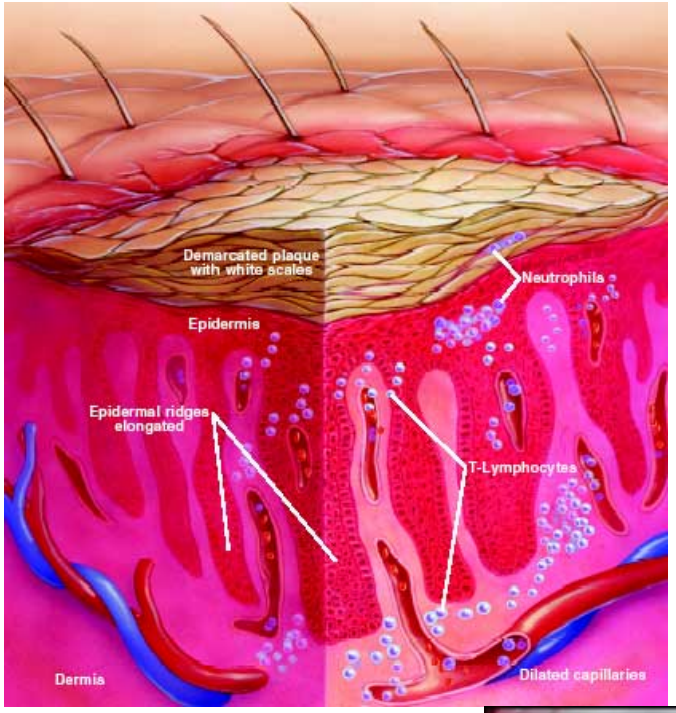
Glowacka A, et al. Scand J Immunol, 2002: 8: 210-7

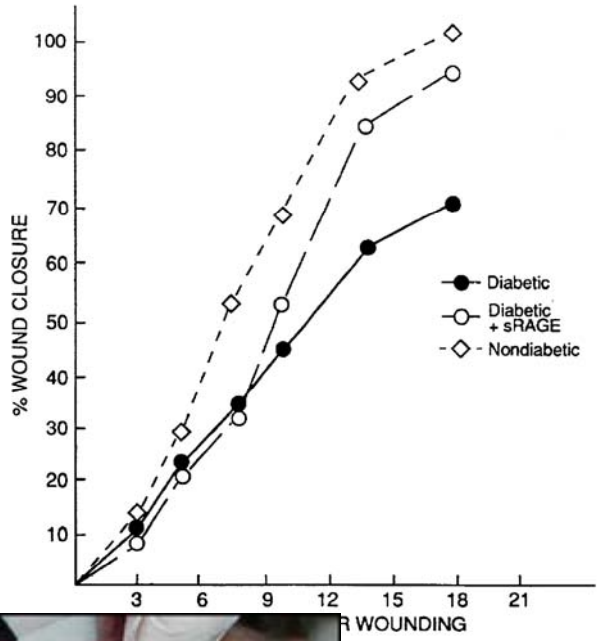
DM ve Nötrofil Fagositoz Aktivitesi

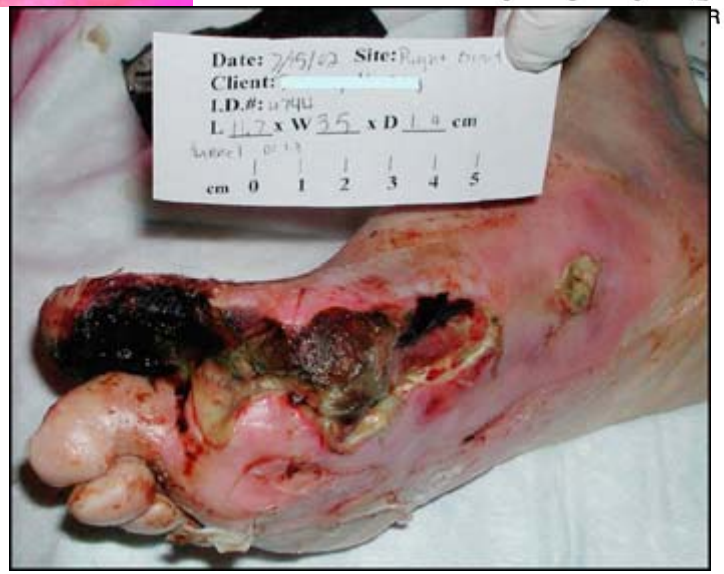


Lin JC, et al. J Clin Endocrin Metabol, 2006; 23: 1210

FIGURE 1


Demarcated plaque with white scales
Epidermis
Epidermal ridges elongated
Neutrophils
T-Lymphocytes
Dilated capillaries
Dermis


% WOUND CLOSURE
100
90
80
70
60
50
40
30
20
10
3
6
9
12
15
18
21
Diabetic
Diabetic + sRAGE
Nondiabetic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İnsülin Tedavisi ile..

- Glukoz kullanımında artış
- Laktat oluşumu ve
- Glikojen oluşumunda artış
- Serbest O_2 radikallerinde azalma
- Migrasyon kapasitesinde artış
- PMN Fagositik indeksde artış

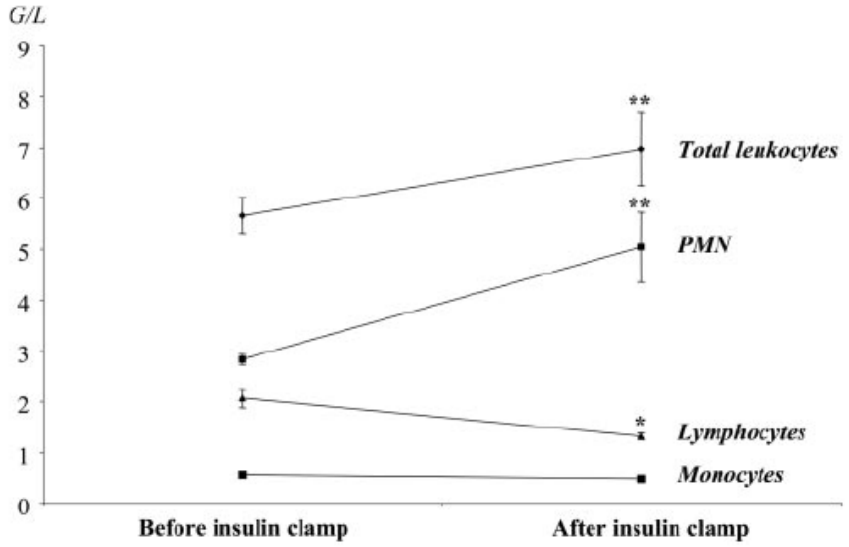


Fig. 1. White blood cell counts before and after insulin clamp in adult healthy subjects. Data are presented as means ± SEM. White blood cell counts are expressed in G/L. Repeated-measure ANOVA + Newman-Keüls test. *, $P < 0.05$, and **, $P < 0.01$, versus before insulin clamp.

Journal of Leukocyte Biology Volume 76, December 2004

Migrasyon kapasitesinde artış

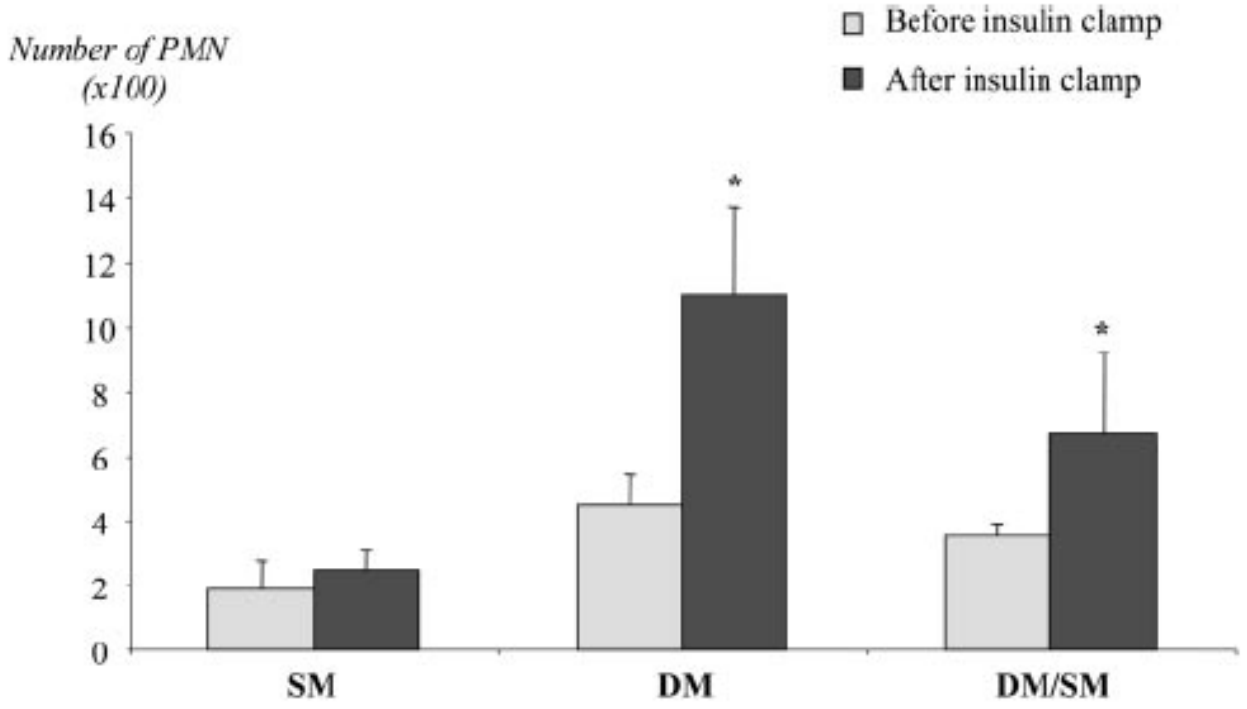


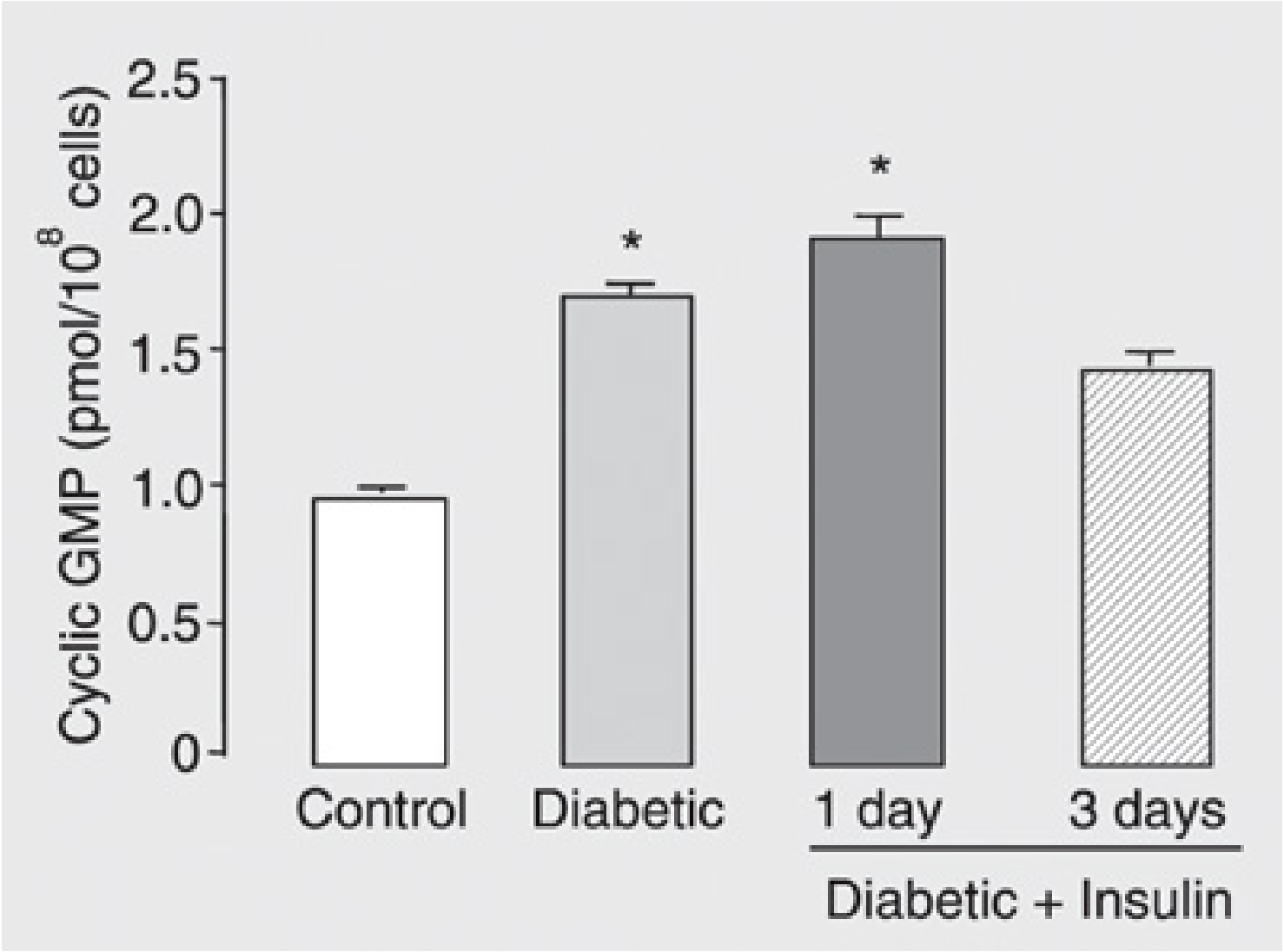
Fig. 3. PMN chemotaxis ability before and after insulin clamp in adult healthy subjects. Data are presented as means ± SEM. PMN chemotaxis is expressed as the spontaneous migration (SM), the directed migration (DM) toward fMLP, and the ratio DM/SM. Repeated-measure ANOVA + Newman-Keüls test. *, $P < 0.05$, versus before insulin clamp.

Fagositik İndeks kapasitesinde artış

TABLE 3. PMN Phagocytosis Indexes before and after Insulin Clamp in Adult Healthy Subjects

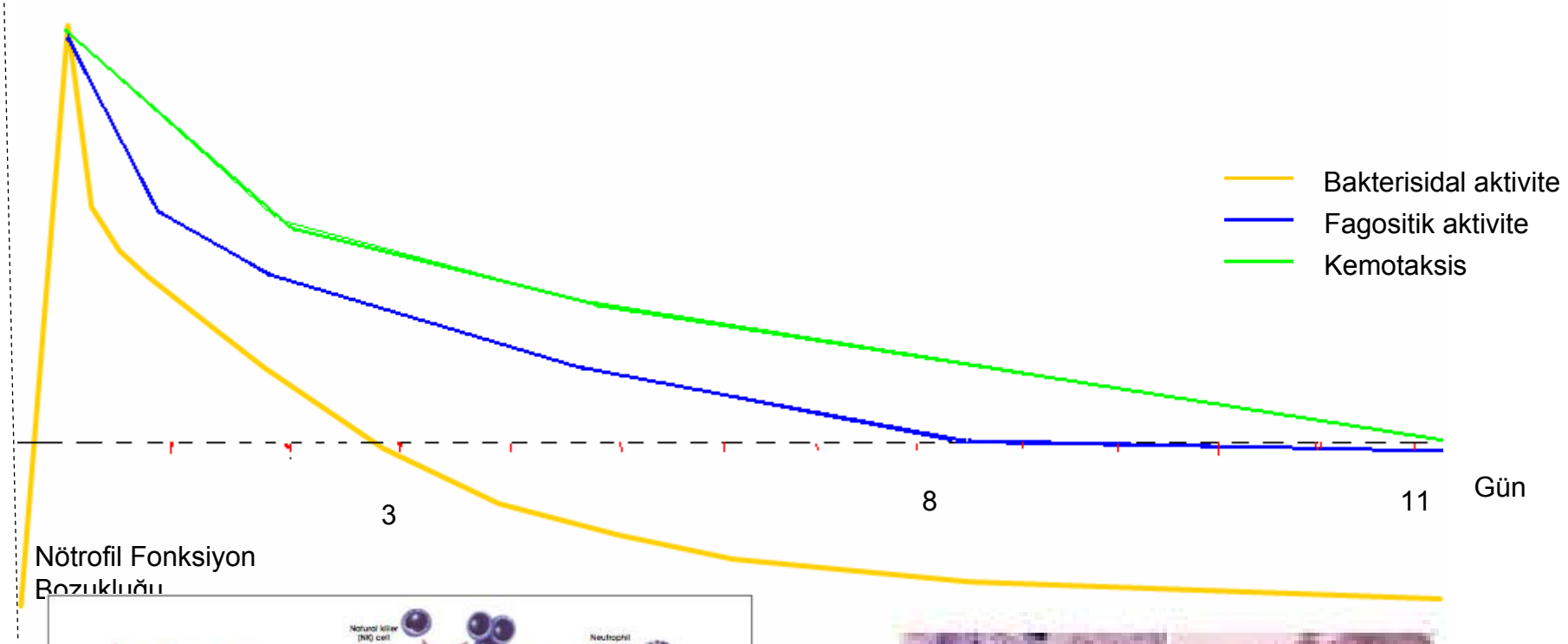
	Before insulin clamp	After insulin clamp
% of Phagocytic PMN[a]	87.7 ± 3.6	90.9 ± 3.6*
Phagocytosis index (AU)[b]	5.40 ± 1.02	6.96 ± 1.24**

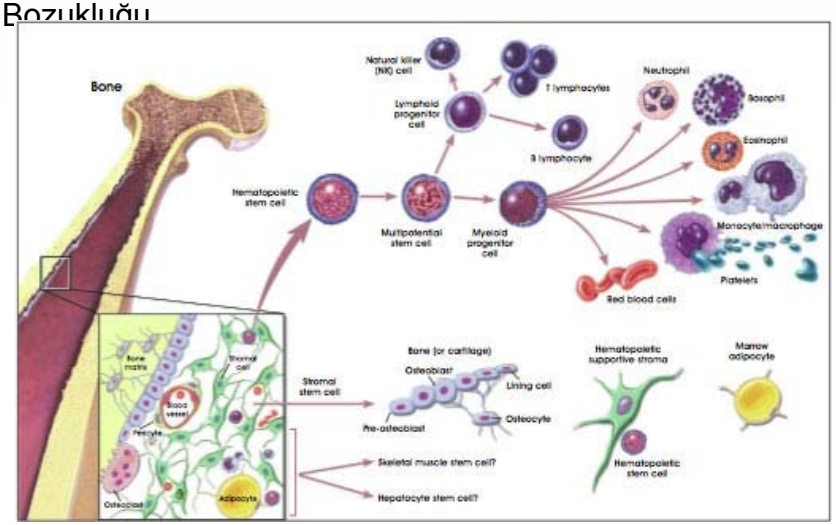
Data are presented as means ± SEM; repeated-measure ANOVA + Newman-Keüls test. * $P < 0.05$ and ** $P < 0.01$ versus before insulin clamp. [a] Percentage of PMN able to ingest OZ particles. [b] Arbitrary unit (AU).

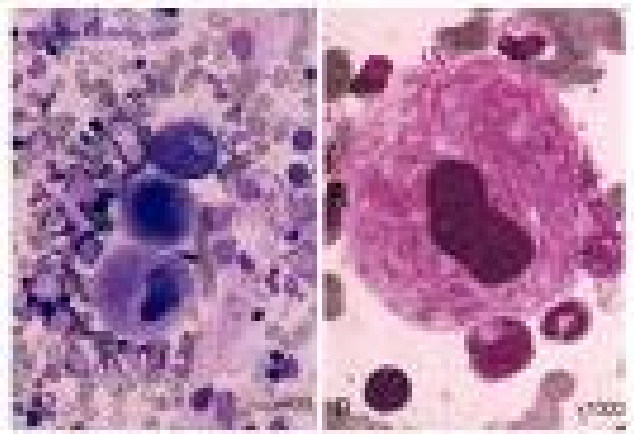


Cyclic GMP levels in neutrophils from diabetic rats, diabetic rats treated with insulin, and matching controls. Rats were rendered diabetic by the injection of alloxan (42 mg/kg, *iv*) 10 days before. Insulin (NPH, 2 IU/day, *sc*) was given for 1 or 3 days before testing. Glycogen-elicited peritoneal neutrophils from 3 to 4 rats were pooled, each animal yielding approximately 1 x 108 cells. Data are reported as pmol cyclic GMP per 108 cells, as mean ± SEM of 3 independent experiments (9 to 12 animals in each group). *P < 0.001 compared to control (ANOVA followed by the Newman-Keuls test).

Kontrollü DM ve Nötrofil Fonksiyonları





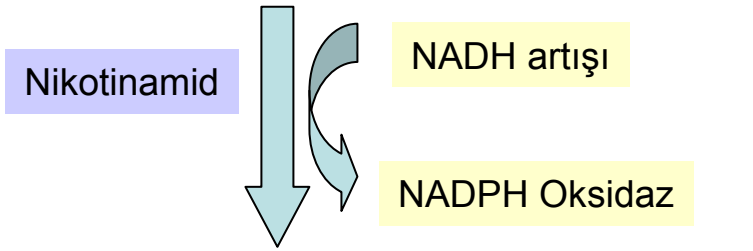


Nicotinamide Effects Oxidative Burst Activity of Neutrophils in Patients with Poorly Controlled Type 2 Diabetes Mellitus

Zeynep Osar,[1] Tülay Samanci,[1] Gülderen Yanikkaya Demirel,[2] Taner Damci,[1] and Hasan Ilkova[1]

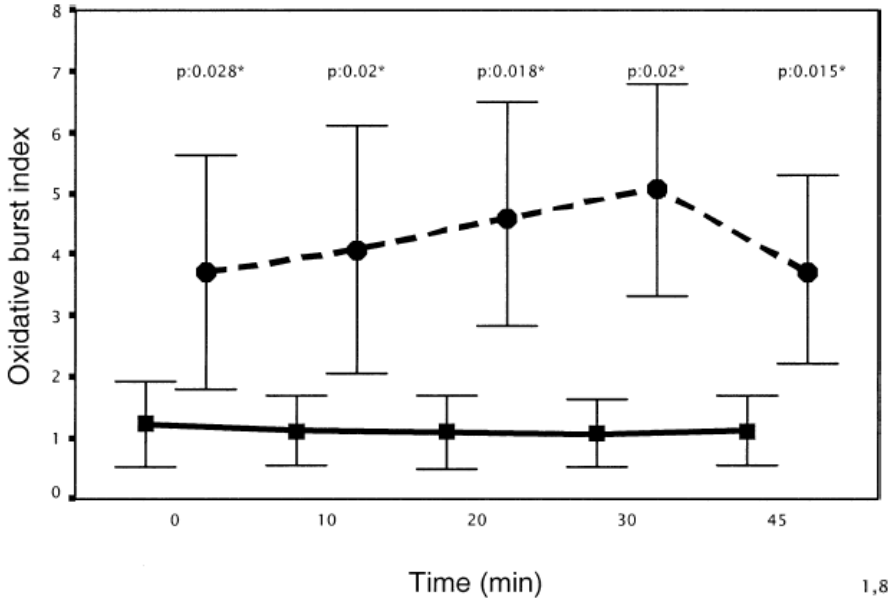
[1]*Department of Internal Medicine, Division of Endocrinology, Metabolism, and Diabetes, Cerrahpasa Medical Faculty, Istanbul University, Istanbul, Turkey*
[2]*Pakize Tarzi Flow Cytometry Laboratory, Memorial Hospital, Istanbul, Turkey*

NADPH Oksidaz akivitesinde bozulma
Süperoksid üretiminde azalma

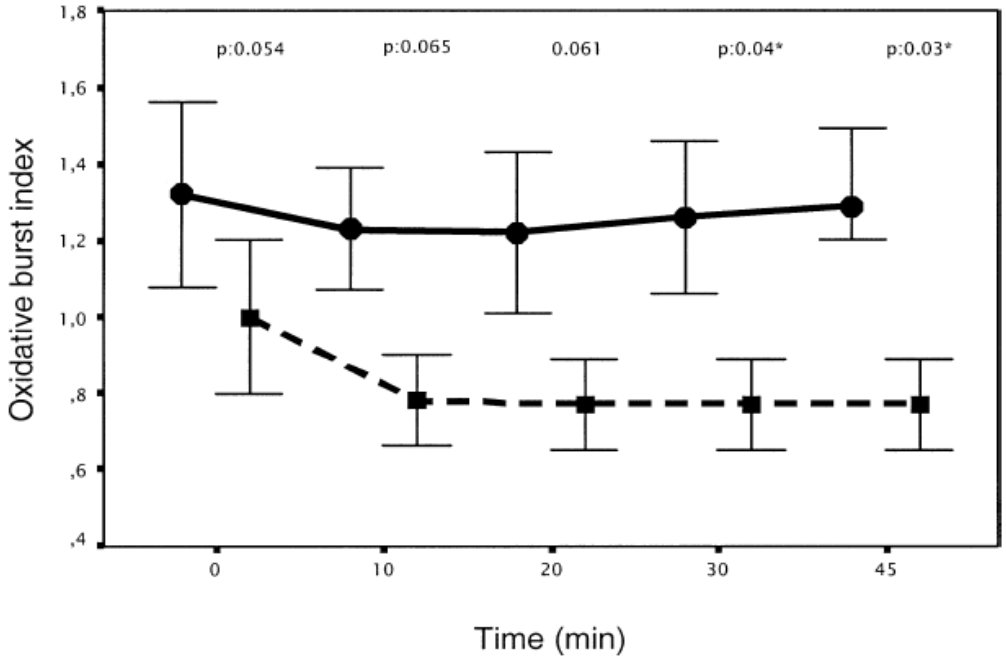


Oksidatif patlama kapasitesinde düzelme

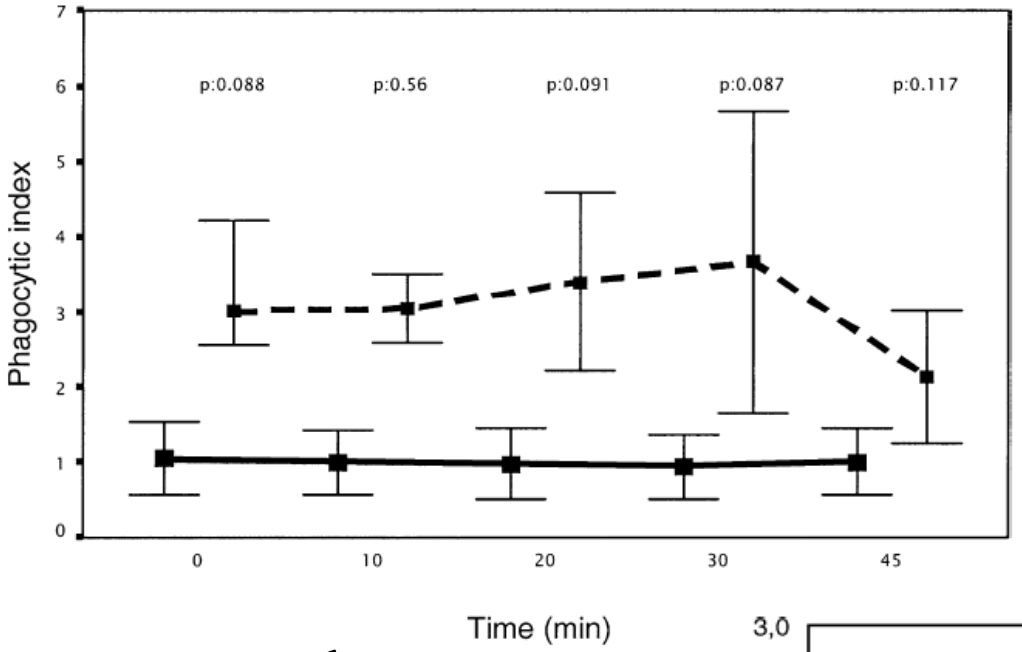
Experimental Diab. Res., 5:155–162, 2004



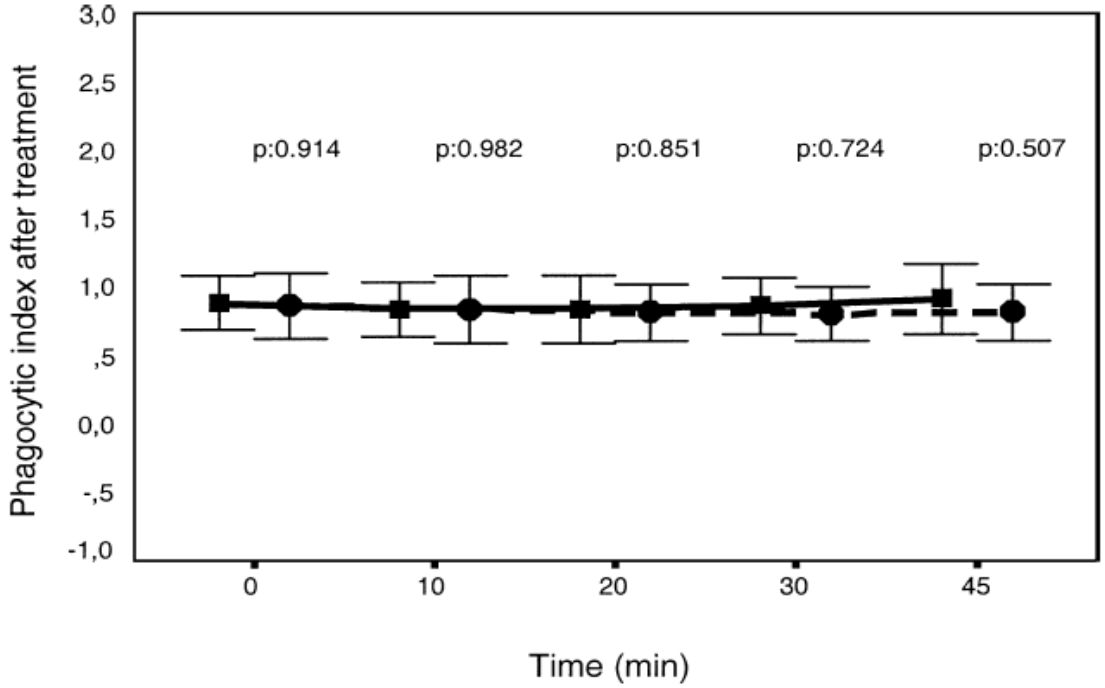
Nikotinamid



Experimental Diab. Res., 5:155–162, 2004



Nikotinamid



Experimental Diab. Res., 5:155–162, 2004

ELSEVIER www.elsevierhealth.com/journals/jinf

Phagocytic activity of neutrophils improves over the course of therapy of diabetic foot infections

Cihan Top [a,*], Senol Yildiz [b], Oral Öncül [c], Tauland Qydedi [b], Adile Çevikbaş [d], Umran Gurer Soyogul [d], Şaban Çavuşlu [b]

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Table 2 Values (mean ± s.d.) of several parameters in studied patients

Variable	Study of subjects before therapy ($n = 38$)	Study of subjects two weeks later ($n = 38$)	Statistical analysis p values
Fasting glucose (mg/dl)	144.3 ± 46.9	138.1 ± 52.5	>0.05
Postprandial glucose (mg/dl)	262.8 ± 69.7	261.6 ± 66.3	>0.05
HbA1c (%)	8.9 ± 2.2	8.5 ± 1.9	<0.05
ESR (mm/h)	52.7 ± 32.9	66.4 ± 28.6	<0.001
CRP (ng/L)	41.4 ± 36.7	17.4 ± 18.2	<0.001

Table 1 The differences in phagocytic index and CRP before and after short-course therapy

	Before therapy	Two weeks later	p^*
Phagocytic index	47.7 ± 11.4	62.5 ± 15.6	<0.001
CRP (mg/L)	41.4 ± 36.7	17.4 ± 18.2	<0.001

*Wilcoxon signed ranks test.

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Effect of the function of polymorphonuclear leukocytes and interleukin-1 beta on wound healing in patients with diabetic foot infections☆

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38 DM ve 28 kontrol
23 İyileşen, 15 iyileşmeyen
9 Amputasyon

Table 1 Demographic and laboratory characteristics of the diabetic patients

Patient's characteristics (mean)	Healing group ($n = 23$)	Non-healing group ($n = 15$)	P
Age	65.6 ± 13.2	66.8 ± 3	0.94
Duration of diabetes (years)	16.43 ± 10.59	23.06 ± 11.20	0.73
Average duration of insulin use	12 ± 4.2	10 ± 3.7	0.72
FBG (mg/dl)	140.2 ± 67.9	148.6 ± 32.9	0.69
Urea (mg/dl)	45.7 ± 21.6	62.3 ± 11.8	0.21
Creatinine (mg/dl)	1.28 ± 0.6	1.39 ± 0.7	0.88
Leukocyte (/μl)	8100 ± 2133	8213 ± 3313	0.34
CRP (mg/L)	14.11 ± 4.33	22.54 ± 5.76	0.05
Erythrocyte sedimentation rate (mm/h)	47.56 ± 25.56	60.63 ± 31.83	0.04
Culture results (CFU/mL)	$3.6 \pm 1.1 \times 10^5$	$9.7 \pm 1.3 \times 10^5$	0.01
Hemoglobin A1C	8.6 ± 2.6	9.3 ± 4.1	0.7
Duration of hospitalization (day)	27 ± 12	43 ± 25	0.03

Chi-squared crosstabs and ANOVA (post hoc comparisons with Student–Newman–Keuls tests were used. $P < 0.5$ considered to be statistically significant.
Results expressed as the mean ± standard deviation. n, Number of patients in each classification. FBGL, fasting blood glucose; HbA1c, hemoglobin A1c; The value of $P < 0.05$ was admitted to be meaningful (χ^2 test, Fisher's exact test and Wilcoxon rank sum test). Culture results show the value of quantitative tissue culture results as CFU/mL.

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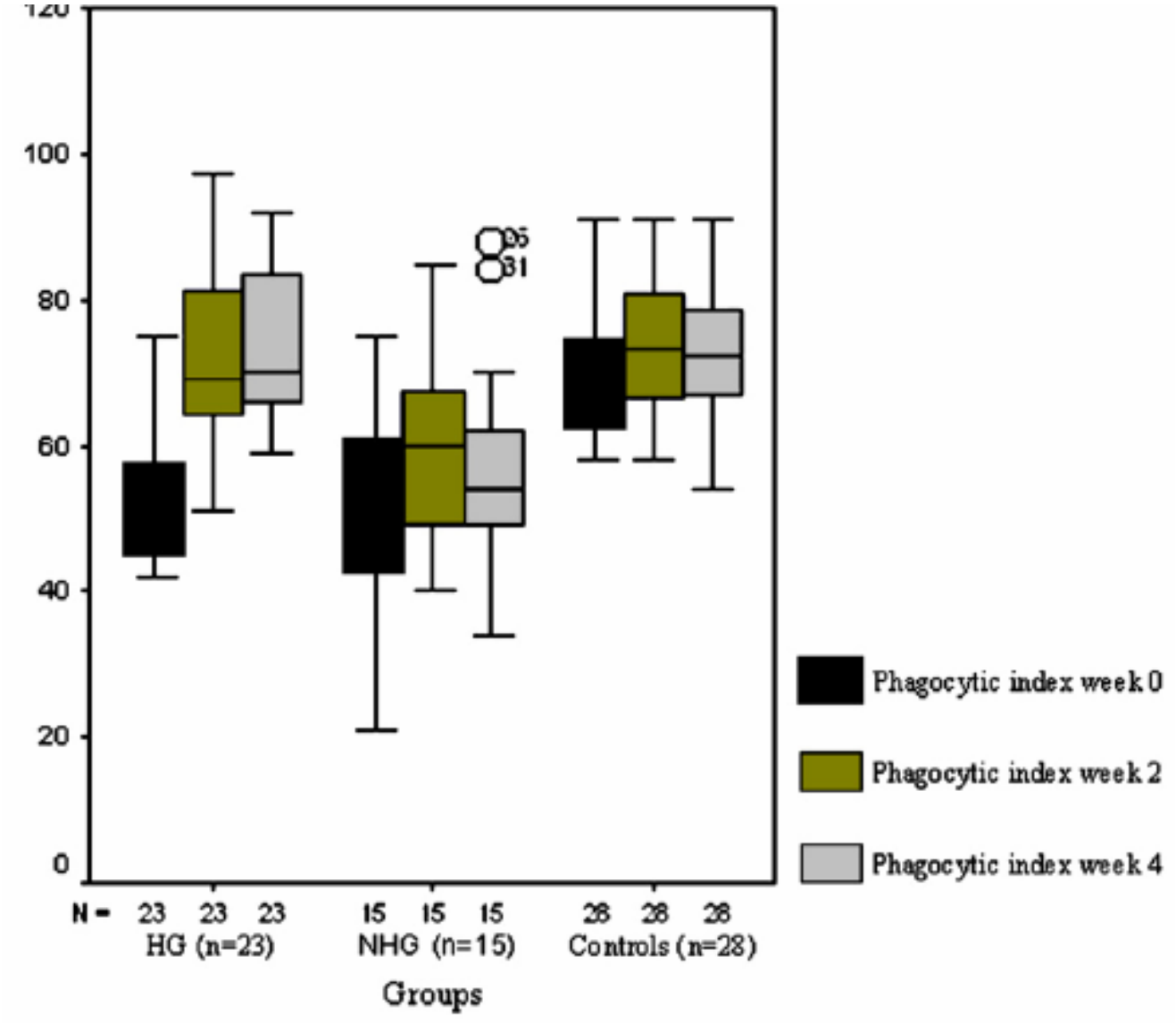
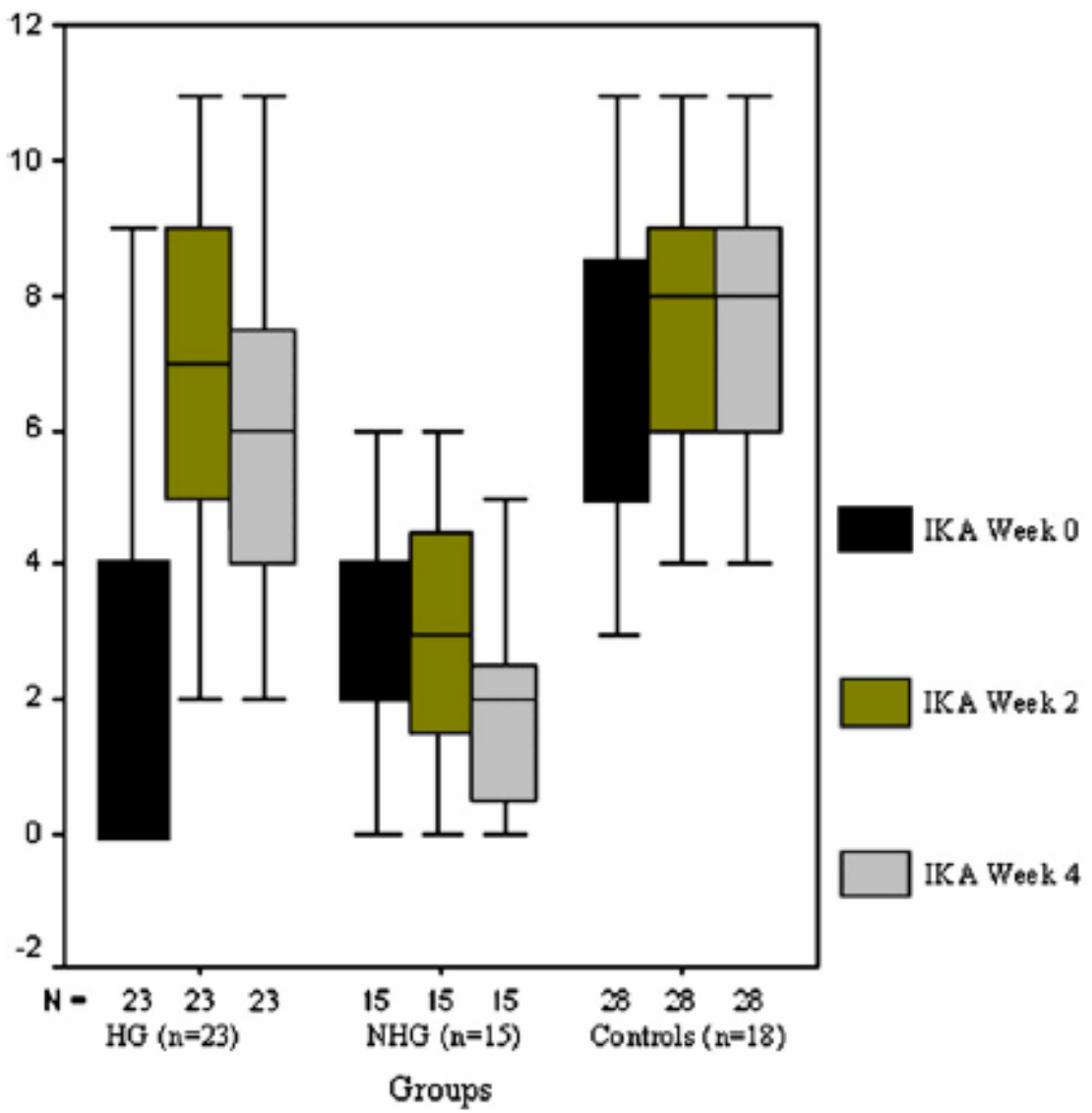
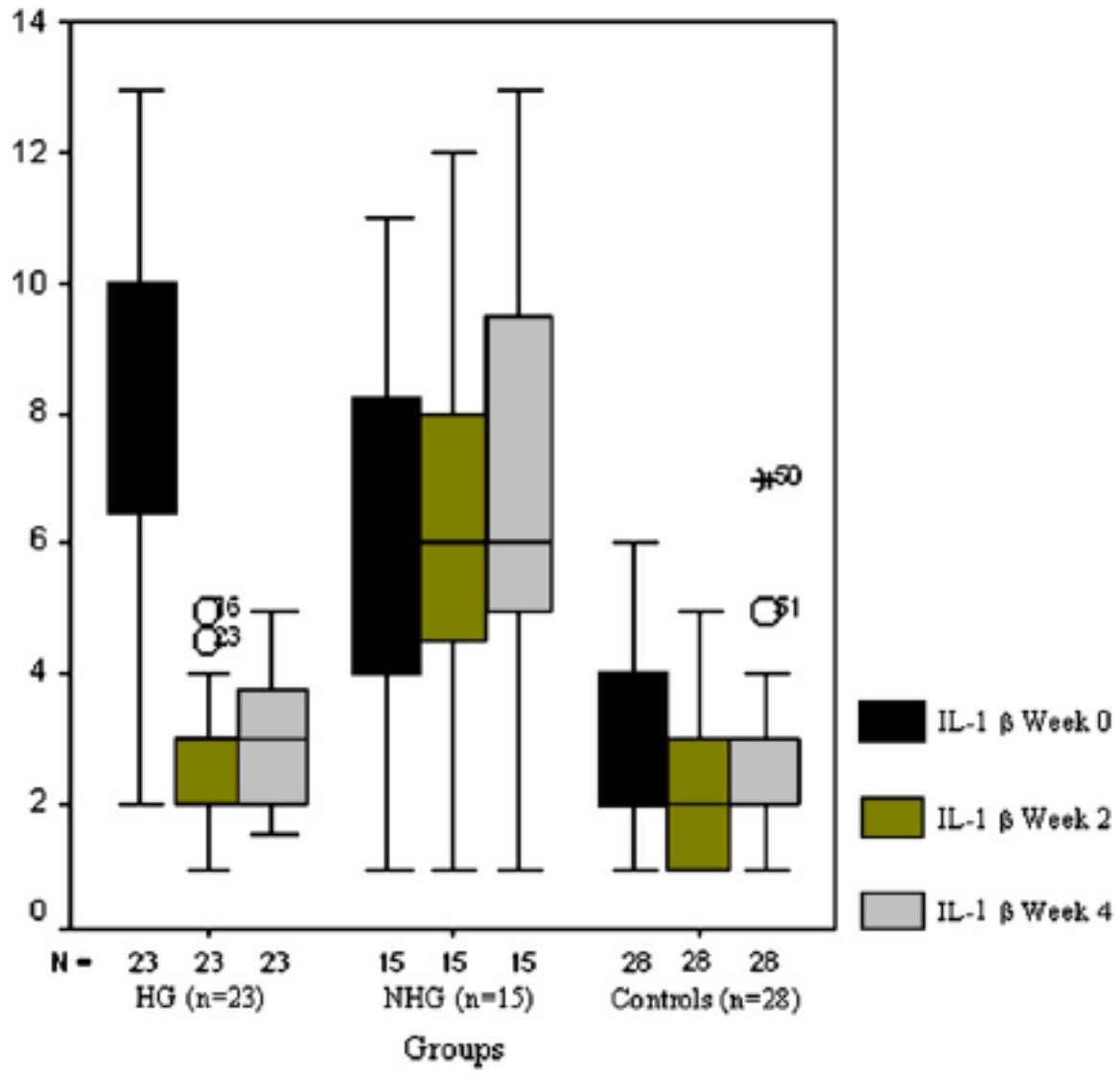
Fagositik İndeks İyileşen Diabetik Ayak İnfeksiyonlarında Daha Yüksek..



Hücre İçi Öldürücü Aktivite İyileşen Diabetik Ayak İnfeksiyonlu Hastalarda Daha Yüksek

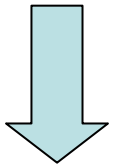


IL-1 Beta İyileşen Diabetik Ayak İnfeksiyonlarında Daha Düşük..



SONUÇ

- Diabetik hastalarda Nötrofil fonksiyon bozukluğu tedaviye yanıtsızlığın öncelikli nedenidir.



•İNSÜLİN

Teşekkürler..