RESEARCH

Interleukin-18 serum level is elevated in type 2 diabetes and latent autoimmune diabetes

Emanuela Zaharieva[1], Zdravko Kamenov[1], Tsvetelina Velikova[2], Adelina Tsakova[3], Yosif El-Darawish[4] and **Haruki Okamura[4]**

[1]Department of Internal Medicine, University Hospital Alexandrovska, Clinic of Endocrinology, Faculty of Medicine, Medical University-Sofia, Sofia, Bulgaria
[2]Department of Clinical Immunology, University Hospital St. Ivan Rilski, Laboratory of Clinical Immunology, Faculty of Medicine, Medical University-Sofia, Sofia, Bulgaria
[3]Department of Clinical Laboratory, University Hospital Alexandrovska, Central Clinical Laboratory, Faculty of Medicine, Medical University-Sofia, Sofia, Bulgaria
[4]Laboratory of Tumor Immunology and Cell Therapy, Hyogo College of Medicine, Hyogo, Japan

Correspondence should be addressed to Z Kamenov: zkamenov@hotmail.com

Abstract

Background: Interleukin-18 (IL-18) is an inflammatory cytokine found to be elevated in obesity, metabolic syndrome and type 2 diabetes (T2D) as a part of the chronic low-grade inflammatory process in these states. The aim of the study was to evaluate the interleukin level in patients with latent autoimmune diabetes of the adults (LADA) in comparison to that in T2D subjects.

Materials and methods: IL-18 was analyzed through enzyme-linked immunosorbent assay in 76 participants with T2D and 24 with LADA and 14 control subjects. Evaluation was also carried out in body mass index (BMI)- and glycemic control-matched diabetic patients.

Results: The serum concentration of IL-18 was higher in patients with T2D (389.04 ± 203.44 pg/mL) and LADA (327.04 ± 144.48 pg/mL) than that in control subjects (219.88 ± 91.03 pg/mL), $P < 0.05$. However, it was not significantly different between both diabetic groups ($P = 0.255$) despite higher IL-6 (4.78 ± 5.84 vs 1.79 ± 0.96 pg/mL, $P < 0.001$) and hs-CRP (2.60 ± 1.70 vs 1.29 ± 1.20 mg/L, $P = 0.002$) level in T2D patients. The results were persistent in BMI-matched subjects with diabetes (IL-18 = 403.48 ± 226.32 vs 329.30 ± 146.30 pg/mL, respectively for T2D and LADA, $P = 0.391$). The correlations in T2D group concerning HDL cholesterol ($r = -0.377$, $P = 0.001$), postprandial glucose ($r = 0.244$, $P = 0.043$), IL-6 ($r = 0.398$, $P < 0.001$) and hs-CRP ($r = 0.427$, $P = 0.001$) were not confirmed in LADA and control subjects.

Conclusion: The IL-18 serum level was higher in T2D and LADA than that in control subjects, but did not differ between both diabetic groups, even when they were BMI matched. Correlations with lipid, glycemic and inflammatory parameters were present in T2D only.

Key Words
- IL-18
- LADA
- type 2 diabetes

Endocrine Connections (2018) **7**, 179–185

Introduction

Interleukin-18 (IL-18), discovered in the late 20th century as an interferon-gamma (IFNγ)-inducing factor (1), is a cytokine that belongs to the IL-1 superfamily (2). It is produced by monocyte/macrophages, endothelial cells, smooth muscle cells, etc. and stimulates the expression of adhesion molecules, chemokine receptors, granulocyte macrophage colony-stimulating factor (GM-CSF), IFNγ, tumor necrosis factor α (TNFα) and interleukin 1β (IL-1β)

http://www.endocrineconnections.org
https://doi.org/10.1530/EC-17-0273

and activates type 1 or type 2 T helper immune response (3, 4). Apart from its place in immune defense against infective pathogens, IL-18 participates in the pathogenesis of atopic, autoimmune and chronic inflammatory diseases (3, 4).

Obesity and type 2 diabetes (T2D) have been associated with a chronic low-grade inflammation (5) that has been implicated as a crucial factor for insulin resistance (6) and also beta-cell dysfunction (7, 8). Patients diagnosed with latent autoimmune diabetes of the adults (LADA) (9), considered by many an indistinguishable part of type 1 diabetes (T1D) (10, 11, 60), usually present with a better metabolic profile and inflammatory markers than T2D, even though optimal glycemic control is usually harder to achieve (12).

Like other pro-inflammatory cytokines (such as IL-1β, TNFα, interleukin 6 (IL-6) (13, 14, 15, 16) and high-sensitivity C-reactive protein (hs-CRP) (17)), elevated IL-18 level is a characteristic of obesity and the metabolic syndrome (18, 19, 20), and is reduced by weight loss (19, 21). The presence of the metabolic syndrome is associated with even higher IL-18 concentration than obesity alone, as has been shown for IL-6, TNFα and CRP (22). It is now well known that adipose tissue is a major contributor to inflammatory cytokine release in these states (23). As with other adipokines (24, 25), adipocytes can produce IL-18 (26, 27), but they do not seem to be the main source of the interleukin – rather it is being predominantly released by infiltrating macrophages (28).

Elevated IL-18 plasma level has been observed *in vivo* in response to acute hyperglycemia in healthy volunteers and subjects with impaired glucose tolerance (29). Patients with newly diagnosed T2D have been shown to have a significantly higher IL-18 level in comparison to non-diabetic subjects (30, 31). This elevation correlates with various factors used in the assessment of the metabolic risk – BMI, waist circumference, HDL cholesterol, triglycerides, blood pressure control, basal insulin, fasting plasma glucose (31, 32, 33) and insulin resistance index (34). Changes in IL-18 seem to be predictive of the risk of prediabetes (35) and T2D (35, 36, 37) in a way that is independent of other markers that reflect the ongoing chronic subclinical inflammation (37). They also contribute to determining the cardiovascular risk in the presence of a metabolic syndrome (38).

The aim of the current study was to compare the serum level of IL-18 between patients with T2D and subjects who meet the criteria for LADA (39) and to estimate its association with weight, glycemic and lipid control, as well as that of the inflammatory markers IL-6 and hs-CRP within both groups.

Subjects and methods

One hundred patients with diabetes were enrolled in this study – 76 patients with T2D and 24 with LADA and 14 control participants. The protocol of the study was in accordance with the declaration of Helsinki (40) and was approved by the ethics committee of Medical University-Sofia. All participants have signed an informed consent. Patients with LADA were enrolled if medical history included all of the following criteria: (1) onset at the age of 35 years or over; (2) presence of positive diabetes-associated autoantibodies, such as islet-cell cytoplasm autoantibodies (ICA), glutamic acid decarboxylase autoantibodies (GAD65A) (41), insulinoma-associated-2 antibodies – tyrosine phosphatase-associated (IA-2A) (42), zinc transporter 8 autoantibodies (ZnT8A) (43); (3) no need for insulin treatment for at least 6 months after diagnosis (39). All participants were assayed for GAD65A within the study as well (Supplementary Table 1, see section on supplementary data given at the end of this article).

The control groups consisted of healthy volunteers with a BMI <30 kg/m^2. Presence of the metabolic syndrome was assessed through the International Diabetes Federation criteria (44).

Blood samples were obtained after an overnight fasting state. Lipid profile, fasting plasma glucose (FPG) and 2-h post standard lunch meal postprandial plasma glucose (PPG), HbA1c were assessed by standard techniques in the Central Laboratory of the University hospital, which is the referent hospital for Bulgaria. IL-6 was analyzed by electro-chemiluminescence immunoassay on Roche Elecsys 2010 (Roche Diagnostics GmbH) and hs-CRP by particle enhanced immunoturbidimetric assay on Cobas Integra 400 Plus (Roche Diagnostics GmbH) with a lower detection limit of 1.5 pg/mL and 0.15 mg/L, respectively. IL-18 was analyzed by enzyme-linked immunosorbent assay (ELISA) (Medical & Biological Laboratories Co., Ltd, Nagoya, Japan) with a detection limit of 12.5 pg/mL. GAD65A were assayed by ELISA (Euroimmune Medizinische Labordiagnostika AG, Lübeck, Germany) with a diagnostic cut-off at 10 IU/mL and a sensitivity and specificity, evaluated in the 2005 Diabetes Autoantibody Standardization Program workshop, of 92% and 98%, respectively.

http://www.endocrineconnections.org
https://doi.org/10.1530/EC-17-0273

Statistical analysis

Statistical analysis was performed with SPSS 21 statistics package. Comparison of data between independent samples was analyzed through the Mann–Whitney *U* test. Spearman's rank correlation coefficient was computed for correlations. Fisher's exact test was used for assessing differences in frequencies between groups. *P* values less than 0.05 were considered significant.

Results

Subjects' characteristics are detailed in Table 1. Patients from both diabetic groups had a significantly higher serum level of IL-18 than the control participants. Taking into consideration the significant difference in gender distribution between T2D subjects and control participants, re-evaluation of the serum level only in women from these groups confirmed a significantly higher serum concentration of the IL-18 in T2D ($n=43$) than in control ($n=13$) participants (400±197 vs 222±94 pg/mL, $P<0.001$).

Despite the subjects with T2D and LADA having significantly different anthropometric parameters and glycemic control, and the T2D group having a higher IL-6 and hs-CRP level, the IL-18 level was not significantly different between both diabetic groups. Analysis between BMI-matched diabetic patients, who still differed in some parameters used in the assessment of metabolic risk (44) but had similar glycemic control, showed that the IL-6 and hs-CRP, although not significantly for the latter, remained higher in T2D group. The difference in the level of IL-18 was still not significant between both diabetic groups (Table 1).

Comparison in IL-18 level between BMI-matched T2D ($n=8$) and control ($n=13$) participants (BMI=22.51±1.08 vs 21.47±1.73 kg/m^2, $P=0.147$; WC=82±8 vs 78±8 cm, $P=0.50$) demonstrated a persistent higher level of the cytokine in the diabetic than that in control subjects (IL-18=430±224 vs 218±94 pg/mL, $P=0.007$).

This was also observed (IL-18=326±135 vs 220±91 pg/mL, $P=0.027$) between the LADA cohort ($n=14$) and control participants ($n=14$) when anthropometric parameters were not significantly different (BMI=22.63±2.40 vs 21.72±1.91 kg/m^2, $P=0.214$, WC=83±8 vs 77±8 cm, $P=0.054$).

The correlation analysis showed significant findings in the T2D group concerning relationship between the IL-18 level and glycemic and lipid control, but not

Table 1 Characteristics of participants.

	T2D ($n=76$)	LADA ($n=24$)	*P*	Controls ($n=14$)	*P**	*P***	T2D ($n=28$)[#]	LADA ($n=20$)[#]	*P*
Age (years)	54±7	53±8	0.558	53±4	0.416	0.940	54 ±8	52±8	0.402
Gender (w/m)	43/33	16/8	0.478	13/1	0.014	0.067	14/14	14/6	0.237
Diabetes duration (years)	4±3	3±2	0.282	–	–	–	4±4	4±2	0.983
MS (+/–)	67/9	6/18	<0.001	0/14	<0.001	0.067	19/9	3/17	<0.001
BMI (kg/m^2)	33.10±6.62	26.04±5.05	<0.001	21.72±1.91	<0.001	0.003	26.29±2.98	24.38±3.44	0.055
WC (cm)	104±14	89±14	<0.001	77±8	<0.001	0.003	92±9	85±9	0.013
SBP (mmHg)	136±19	117±13	<0.001	109±10	<0.001	0.044	134±18	116±13	0.001
DBP (mmHg)	84±10	76±9	0.001	71±8	<0.001	0.060	83±11	75±9	0.013
FPG (mmol/L)	8.2±2.9	8.2±3.1	0.884	5.02±0.39	<0.001	<0.001	9.1±3.4	7.9±3.2	0.188
PPG (mmol/L)	8.4±3.2	10.4±4.1	0.041	–	–	–	9.4±3.4	10.2±4.3	0.638
HbA1c (%)	7.9±2.0	9.2±1.4	0.001	–	–	–	8.7±2.2	9.3±1.4	0.228
TC (mmol/L)	5.15±1.07	5.42±1.45	0.689	4.91±0.74	0.503	0.414	5.26±1.23	5.29±1.30	0.788
LDL-C (mmol/L)	3.01±0.80	2.74±1.42	0.163	2.22±0.85	0.145	0.727	2.94±0.95	2.57±1.16	0.500
HDL-C (mmol/L)	1.27±0.34	1.73±0.51	<0.001	1.67±0.65	0.224	0.694	1.31±0.39	1.81±0.51	0.002
TG (mmol/L)	1.80±0.99	1.46±1.01	0.011	1.11±1.12	<0.001	0.059	1.74±0.82	1.20±0.69	0.006
hs-CRP (mg/L)	2.60±1.70	1.29±1.20	0.002	0.79±1.04	<0.001	0.076	1.97±1.66	1.17±1.25	0.125
IL-6 (pg/mL)	4.78±5.84	1.79±0.96	<0.001	1.57±0.26	<0.001	0.410	4.42±5.05	1.58±0.34	0.002
IL-18 (pg/mL)	389±203	327±144	0.255	220±91	<0.001	0.013	403±226	329±146	0.391

Results are expressed as mean±S.D.
*Compared with T2D; **compared with LADA; [#]characteristics within groups with BMI-matched participants.
BMI, body mass index; DBP, diastolic blood pressure; FPG, fasting plasma glucose; HDL-C high density lipoprotein cholesterol; Hs-CRP, high-sensitivity C-reactive protein; IL-6, interleukin 6; IL-18, interleukin-18; LADA, latent autoimmune diabetes of the adults; LDL-C, low density lipoprotein cholesterol; MS, metabolic syndrome; PPG, postprandial plasma glucose; SBP, systolic blood pressure; T2D, type 2 diabetes; TC, total cholesterol; TG, triglycerides; WC, waist circumference.

Table 2 Correlation coefficients between IL-18 and clinical, metabolic and inflammatory parameters.

	T2D ($n=76$)		**LADA** ($n=24$)		**Controls** ($n=14$)		**T2D** ($n=28$)[#]		**LADA** ($n=20$)[#]	
	r	*P*	*r*	*P*	*r*	*P*	*r*	*P*	*r*	*P*
BMI (kg/m^2)	0.015	0.897	0.210	0.325	0.004	0.988	0.240	0.219	0.328	0.158
WC (cm)	0.073	0.532	0.297	0.169	0.064	0.827	0.219	0.262	0.379	0.110
SBP (mmHg)	−0.037	0.749	0.289	0.171	0.527	0.053	0.172	0.382	0.228	0.333
DBP (mmHg)	−0.094	0.418	0.133	0.535	0.507	0.064	0.080	0.687	0.067	0.780
FPG (mmol/L)	0.075	0.530	−0.098	0.655	0.483	0.094	0.152	0.449	−0.261	0.280
PPG (mmol/L)	0.244	0.043	0.027	0.907	–	–	0.290	0.151	−0.001	0.997
HbA1c (%)	0.007	0.956	0.168	0.434	–	–	0.059	0.771	0.157	0.508
LDL-C (mmol/L)	−0.141	0.251	0.134	0.563	−0.500	0.667	−0.099	0.645	0.059	0.823
HDL-C (mmol/L)	−0.377	0.001	−0.392	0.079	0.500	0.667	−0.464	0.019	−0.412	0.101
TG (mmol/L)	0.072	0.546	0.301	0.154	−0.172	0.557	0.044	0.829	0.281	0.231
hs-CRP (mg/L)	0.427	0.001	0.113	0.832	−0.241	0.406	0.493	0.017	0.128	0.591
IL-6 (pg/mL)	0.398	<0.001	−0.131	0.541	−0.241	0.406	0.408	0.035	0.020	0.934

[#]Correlations within groups with BMI-matched participants.
BMI, body mass index; DBP, diastolic blood pressure; FPG, fasting plasma glucose; HDL-C, high density lipoprotein cholesterol; Hs-CRP, high-sensitivity C-reactive protein; IL-6, interleukin 6; IL-18, interleukin-18; LADA, latent autoimmune diabetes of the adults; LDL-C, low density lipoprotein cholesterol; PPG, postprandial plasma glucose; SBP, systolic blood pressure; T2D, type 2 diabetes; TG, triglycerides; WC, waist circumference.

obesity, assessed by both BMI and waist circumference. However, IL-18 correlated positively with both IL-6 and hs-CRP serum concentrations only in T2D group. Results concerning IL-6, hs-CRP and HDL cholesterol were similar even when analysis included subjects with lower BMI. None of these results was observed in the LADA and control groups (Table 2).

Discussion

Chronic low-grade inflammation is part of the pathogenesis of T2D (5), while LADA seems to be characterized with lower levels of inflammatory cytokines like IL-6 and TNFα (45). Serine phosphorylation of insulin receptor substrate (IRS), inhibition of the tyrosine kinase activity of the insulin receptor, ubiquitination and degradation of both IRS1 and IRS2, decreased transcription of IRS1 mRNA are some of the mechanisms involved in the reduction of insulin sensitivity in hepatocytes, adipocytes and muscles by the increased level of inflammatory cytokines (46). Whether IL-18 contributes to insulin resistance (47) is still not well understood although it has been associated with signal transducer and activator of transcription (STAT) signaling (48). Although insulin resistance cannot be excluded in some cases of autoimmune diabetes (49, 50), the metabolic syndrome and associated chronic inflammation are less often observed than in T2D (12, 45).

Unchanged serum concentration of IL-18 in LADA in comparison to T2D could be attributed to the stimulatory effect of hyperglycemia (51), and also to the involvement of the cytokine in autoimmune diseases, including diabetes mellitus (52). Increased level of IL-18 has been observed in some autoimmune diseases as multiple sclerosis, myasthenia gravis, systemic lupus erythematosus, Sjögren syndrome and rheumatoid arthritis, in both experimental models and clinical practice (52). It has been found to be elevated in T1D as well and its higher expression in the islets could be involved in the autoimmune response against beta-cells (51, 53). We have not been able to find data about the serum concentration of IL-18 in LADA in the literature, but being considered a part of the spectrum of T1D (10, 11, 60), we cannot exclude that its level in circulation is influenced by autoimmune processes in the islets.

It has been previously shown that IL-6 and TNFα levels differ significantly in subjects with T2D and patients with late-onset diabetes and positive anti-GAD65 autoantibodies (45) and we have also confirmed different inflammatory parameters in both states (54). Despite the expected higher serum concentrations of IL-6 and hs-CRP in T2D patients when compared with LADA, the level of another potent pro-inflammatory cytokine, namely IL-18, remained unchanged between participants from both groups. The current positive correlation between IL-18 and IL-6, together with hs-CRP, is in line with a previously observed association between them and the metabolic syndrome (22). The latter was much more prevalent in T2D even in BMI-matched participants in the current study. Although correlations of IL-18 with HbA1c, PPG and HDL cholesterol have been previously described in T1D (55), here they were not confirmed in subjects with LADA. One reason for the lack of association between lipid, glycemic and

inflammatory parameters in these participants could be the lower prevalence of the metabolic syndrome, as the association of IL-18 with the latter seems to be stronger than that with obesity (22).

Another possible explanation concerns a dominant role of IL-18 in the autoimmune pathogenesis of LADA and not the insulin resistance-associated chronic low-grade inflammation in this type of diabetes mellitus. Although beta-cell dysfunction in T2D has recently also been associated with insulitis (56), it is currently not clear whether IL-18 contributes to beta-cell apoptosis in T2D as has been demonstrated for other cytokines (57).

Lack of association between IL-18 and the described metabolic and inflammatory parameters could even result from both the metabolic and immunologic factors discussed. It has been shown that LADA could present with a heterogeneous clinical and immunologic characteristics moving it closer to either T1D or T2D (58). A larger cohort of participants with LADA with both phenotypes and related GAD65A titer (59) would clarify the role of IL-18 in metabolic control-associated low-grade chronic inflammation and autoimmune process.

The main limitations of the study are the relatively low number of participants, a higher percentage of the female gender in all groups and the significant difference in the prevalence of the metabolic syndrome between groups, even when they were BMI matched. However, we have not been able to find another study that compares the IL-18 serum level between T2D and LADA, and these results add to current knowledge of the cytokine's place in diabetes mellitus and prompts further investigations of the subject.

Conclusion

The current study showed a higher serum level of IL-18 in patients with T2D and LADA than in the control subjects, but a similar one between both diabetic groups even after matching them for BMI and glycemic control. Correlations with glycemic, lipid and inflammatory parameters were observed in T2D only. Further analysis is needed to distinguish the role of IL-18 in insulin-resistance-associated chronic low-grade inflammation from that in the autoimmune response in LADA.

Supplementary data
This is linked to the online version of the paper at https://doi.org/10.1530/EC-17-0273.

Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research reported.

Funding
The study was financially supported by Medical University-Sofia with grant number 6-D/2014.

Acknowledgements
IL-18 ELISA kits were provided by Hirakata Ryōikuen Osaka.

References

1 Okamura H, Tsutsi H, Komatsu T, Yutsudo M, Hakura A, Tanimoto T, Torigoe K, Okura T, Nukada Y, Hattori K, *et al*. Cloning of a new cytokine that induces IFN-gamma production by T cells. *Nature* 1995 **378** 88–91. (https://doi.org/10.1038/378088a0)
2 Dinarello CA. Interleukin-18, a proinflammatory cytokine. *European Cytokine Network* 2000 **11** 483–486.
3 Dinarello CA. The IL-1 family and inflammatory diseases. *Clinical and Experimental Rheumatology* 2002 **20** S1–S13.
4 Nakanishi K, Yoshimoto T, Tsutsui H & Okamura H. Interleukin-18 is a unique cytokine that stimulates both Th1 and Th2 responses depending on its cytokine milieu. *Cytokine and Growth Factor Reviews* 2001 **12** 53–72. (https://doi.org/10.1016/S1359-6101(00)00015-0)
5 Hameed I, Masoodi SR, Mir SA, Nabi M, Ghazanfar K & Ganai BA. Type 2 diabetes mellitus: from a metabolic disorder to an inflammatory condition. *World Journal of Diabetes* 2015 **6** 598–612. (https://doi.org/10.4239/wjd.v6.i4.598)
6 Ye J. Mechanisms of insulin resistance in obesity. *Frontiers of Medicine* 2013 **7** 14–24. (https://doi.org/10.1007/s11684-013-0262-6)
7 Ehses JA, Perren A, Eppler E, Ribaux P, Pospisilik JA, Maor-Cahn R, Gueripel X, Ellingsgaard H, Schneider MK, Biollaz G, *et al*. Increased number of islet-associated macrophages in type 2 diabetes. *Diabetes* 2007 **56** 2356–2370. (https://doi.org/10.2337/db06-1650)
8 Eguchi K, Manabe I, Oishi-Tanaka Y, Ohsugi M, Kono N, Ogata F, Yagi N, Ohto U, Kimoto M, Miyake K, *et al*. Saturated fatty acid and TLR signaling link β cell dysfunction and islet inflammation. *Cell Metabolism* 2012 **15** 518–533. (https://doi.org/10.1016/j.cmet.2012.01.023)
9 Zimmet PZ, Tuomi T, Mackay IR, Rowley MJ, Knowles W, Cohen M & Lang DA. Latent autoimmune diabetes mellitus in adults (LADA): the role of antibodies to glutamic acid decarboxylase in diagnosis and prediction of insulin dependency. *Diabetes Medicine* 1994 **11** 299–303. (https://doi.org/10.1111/j.1464-5491.1994.tb00275.x)
10 Redondo MJ. LADA: time for a new definition. *Diabetes* 2013 **62** 339–340. (https://doi.org/10.2337/db12-1171)
11 Gale EA. Latent autoimmune diabetes in adults: a guide for the perplexed. *Diabetologia* 2005 **48** 2195–2199. (https://doi.org/10.1007/s00125-005-1954-5)
12 Hawa MI, Thivolet C, Mauricio D, Alemanno I, Cipponeri E, Collier D, Hunter S, Buzzetti R, de Leiva A, Pozzilli P, *et al*. Metabolic syndrome and autoimmune diabetes: action LADA 3. *Diabetes Care* 2009 **32** 160–164. (https://doi.org/10.2337/dc08-1419)
13 Moschen AR, Molnar C, Enrich B, Geiger S, Ebenbichler CF & Tilg H. Adipose and liver expression of interleukin (IL)-1 family members in morbid obesity and effects of weight loss. *Molecular Medicine* 2011 **17** 840–845. (https://doi.org/10.2119/molmed.2010.00108)
14 Kern PA, Saghizadeh M, Ong JM, Bosch RJ, Deem R & Simsolo RB. The expression of tumor necrosis factor in human adipose tissue. Regulation by obesity, weight loss, and relationship to lipoprotein

lipase. *Journal of Clinical Investigation* 1995 **95** 2111–2119. (https://doi.org/10.1172/JCI117899)
15 Bastard JP, Jardel C, Bruckert E, Blondy P, Capeau J, Laville M, Vidal H & Hainque B. Elevated levels of interleukin 6 are reduced in serum and subcutaneous adipose tissue of obese women after weight loss. *Journal of Clinical Endocrinology and Metabolism* 2000 **85** 3338–3342. (https://doi.org/10.1210/jcem.85.9.6839)
16 Jung SH, Park HS, Kim KS, Choi WH, Ahn CW, Kim BT, Kim SM, Lee SY, Ahn SM, Kim YK, *et al*. Effect of weight loss on some serum cytokines in human obesity: increase in IL-10 after weight loss. *Journal of Nutritional Biochemistry* 2008 **19** 371–375. (https://doi.org/10.1016/j.jnutbio.2007.05.007)
17 Tchernof A, Nolan A, Sites CK, Ades PA & Poehlman ET. Weight loss reduces C-reactive protein levels in obese postmenopausal women. *Circulation* 2002 **105** 564–569. (https://doi.org/10.1161/hc0502.103331)
18 Escobar-Morreale HF, Botella-Carretero JI, Villuendas G, Sancho J & San Millán JL. Serum interleukin-18 concentrations are increased in the polycystic ovary syndrome: relationship to insulin resistance and to obesity. *Journal of Clinical Endocrinology and Metabolism* 2004 **89** 806–811. (https://doi.org/10.1210/jc.2003-031365)
19 Esposito K, Pontillo A, Ciotola M, Di Palo C, Grella E, Nicoletti G & Giugliano D. Weight loss reduces interleukin-18 levels in obese women. *Journal of Clinical Endocrinology and Metabolism* 2002 **87** 3864–3866. (https://doi.org/10.1210/jcem.87.8.8781)
20 Angelova P, Kamenov Z, Tsakova A, El-Darawish Y & Okamura H. Interleukin-18 and testosterone levels in men with metabolic syndrome. *Aging Male* [epub]. (https://doi.org/10.1080/13685538.2017.1401993)
21 Tajik N, Keshavarz SA, Masoudkabir F, Djalali M, Sadrzadeh-Yeganeh HH, Eshraghian MR, Chamary M, Ahmadivand Z, Yazdani T & Javanbakht MH. Effect of diet-induced weight loss on inflammatory cytokines in obese women. *Journal of Endocrinological Investigation* 2013 **36** 211–215. (https://doi.org/10.3275/8465)
22 Van Guilder GP, Hoetzer GL, Greiner JJ, Stauffer BL & Desouza CA. Influence of metabolic syndrome on biomarkers of oxidative stress and inflammation in obese adults. *Obesity* 2006 **14** 2127–2131. (https://doi.org/10.1038/oby.2006.248)
23 Gregor MF & Hotamisligil GS. Inflammatory mechanisms in obesity. *Annual Review of Immunology* 2011 **29** 415–445. (https://doi.org/10.1146/annurev-immunol-031210-101322)
24 Weisberg SP, McCann D, Desai M, Rosenbaum M, Leibel RL & Ferrante AW Jr. Obesity is associated with macrophage accumulation in adipose tissue. *Journal of Clinical Investigation* 2003 **112** 1796–1808. (https://doi.org/10.1172/JCI19246)
25 Xu H, Barnes GT, Yang Q, Tan G, Yang D, Chou CJ, Sole J, Nichols A, Ross JS, Tartaglia LA, *et al*. Chronic inflammation in fat plays a crucial role in the development of obesity-related insulin resistance. *Journal of Clinical Investigation* 2003 **112** 1821–1830. (https://doi.org/10.1172/JCI19451)
26 Skurk T, Kolb H, Muller-Scholze S, Rohrig K, Hauner H & Herder C. The proatherogenic cytokine interleukin-18 is secreted by human adipocytes. *European Journal of Endocrinology* 2005 **152** 863–868. (https://doi.org/10.1530/eje.1.01897)
27 Wood IS, Wang B, Jenkins JR & Trayhurn P. The pro-inflammatory cytokine IL-18 is expressed in human adipose tissue and strongly upregulated by TNFalpha in human adipocytes. *Biochemical and Biophysical Research Communications* 2005 **337** 422–429. (https://doi.org/10.1016/j.bbrc.2005.09.068)
28 Fain JN, Tichansky DS & Madan AK. Most of the interleukin 1 receptor antagonist, cathepsin S, macrophage migration inhibitory factor, nerve growth factor, and interleukin 18 release by explants of human adipose tissue is by the non-fat cells, not by the adipocytes. *Metabolism* 2006 **55** 1113–1121. (https://doi.org/10.1016/j.metabol.2006.04.008)
29 Esposito K, Nappo F, Marfella R, Giugliano G, Giugliano F, Ciotola M, Quagliaro L, Ceriello A & Giugliano D. Inflammatory cytokine concentrations are acutely increased by hyperglycemia in humans: role of oxidative stress. *Circulation* 2002 **106** 2067–2072. (https://doi.org/10.1161/01.CIR.0000034509.14906.AE)
30 Esposito K, Nappo F, Giugliano F, Di Palo C, Ciotola M, Barbieri M, Paolisso G & Giugliano D. Cytokine milieu tends toward inflammation in type 2 diabetes. *Diabetes Care* 2003 **26** 1647. (https://doi.org/10.2337/diacare.26.5.1647)
31 Aso Y, Okumura K, Takebayashi K, Wakabayashi S & Inukai T. Relationships of plasma IL-18 concentrations to hyperhomocysteinemia and carotid intimal-media wall thickness in patients with type 2 diabetes. *Diabetes Care* 2003 **26** 2622–2627. (https://doi.org/10.2337/diacare.26.9.2622)
32 Hung J, McQuillan BM, Chapman CM, Thompson PL & Beilby JP. Elevated interleukin-18 levels are associated with the metabolic syndrome independent of obesity and insulin resistance. *Arteriosclerosis, Thrombosis, and Vascular Biology* 2005 **25** 1268–1273. (https://doi.org/10.1161/01.ATV.0000163843.70369.12)
33 Evans J, Collins M, Jennings C, van der Merwe L, Soderstrom I & Olsson T. The association of interleukin-18 genotype and serum levels with metabolic risk factors for cardiovascular disease. *European Journal of Endocrinology* 2007 **157** 633–640. (https://doi.org/10.1530/EJE-07-0463)
34 Fischer CP, Perstrup LB, Berntsen A, Eskildsen P & Pedersen BK. Elevated plasma interleukin-18 is a marker of insulin-resistance in type 2 diabetic and non-diabetic humans. *Clinical Immunology* 2005 **117** 152–160. (https://doi.org/10.1016/j.clim.2005.07.008)
35 Brahimaj A, Ligthart S, Ghanbari M, Ikram MA, Hofman A, Franco OH, Kavousi M & Dehghan A. Novel inflammatory markers for incident pre-diabetes and type 2 diabetes: the Rotterdam Study. *European Journal of Epidemiology* 2017 **32** 217–226. (https://doi.org/10.1007/s10654-017-0236-0)
36 Hivert MF, Sun Q, Shrader P, Mantzoros CS, Meigs JB & Hu FB. Circulating IL-18 and the risk of type 2 diabetes in women. *Diabetologia* 2009 **52** 2101–2108. (https://doi.org/10.1007/s00125-009-1455-z)
37 Thorand B, Kolb H, Baumert J, Koenig W, Chambless L & Meisinger C. Elevated levels of interleukin-18 predict the development of type 2 diabetes: results from the MONICA/KORA Augsburg Study, 1984–2002. *Diabetes* 2005 **54** 2932–2938. (https://doi.org/10.2337/diabetes.54.10.2932)
38 Trøseid M, Seljeflot I, Hjerkinn EM & Arnesen H. Interleukin-18 is a strong predictor of cardiovascular events in elderly men with the metabolic syndrome: synergistic effect of inflammation and hyperglycemia. *Diabetes Care* 2009 **32** 486–492. (https://doi.org/10.2337/dc08-1710)
39 Brooks-Worrell BM & Palmer JP. *Latent Autoimmune Diabetes in Adults: Immunoendocrinology: Scientific and Clinical Aspects (Contemporary Endocrinology)*, pp 315–330. New York, NY, USA: Humana Press, 2011.
40 World Medical Association. World Medical Association Declaration of Helsinki: ethical principles for medical research involving human subjects. *JAMA* 2013 **310** 2191–2194.
41 Turner R, Stratton I, Horton V, Manley S, Zimmet P, Mackay IR, Shattock M, Bottazzo GF & Holman R. UKPDS 25: autoantibodies to islet-cell cytoplasm and glutamic acid decarboxylase for prediction of insulin requirement in type 2 diabetes. UK Prospective Diabetes Study Group. *Lancet* 1997 **350** 1288–1293. (https://doi.org/10.1016/S0140-6736(97)03062-6)
42 Tiberti C, Giordano C, Locatelli M, Bosi E, Bottazzo GF, Buzzetti R, Cucinotta D, Galluzzo A, Falorni A & Dotta F. Identification of tyrosine phosphatase 2(256–760) construct as a new, sensitive marker for the detection of islet autoimmunity in type 2 diabetic patients: the non-insulin requiring autoimmune diabetes (NIRAD) study 2. *Diabetes* 2008 **57** 1276–1283. (https://doi.org/10.2337/db07-0874)

43 Lampasona V, Petrone A, Tiberti C, Capizzi M, Spoletini M, di Pietro S, Songini M, Bonicchio S, Giorgino F, Bonifacio E, *et al*. Zinc transporter 8 antibodies complement GAD and IA-2 antibodies in the identification and characterization of adult-onset autoimmune diabetes. *Diabetes Care* 2010 **33** 104–108. (https://doi.org/10.2337/dc08-2305)

44 International Diabetes Federation. *The IDF Consensus Worldwide Definition of the Metabolic Syndrome*. Brussels, Belgium: IDF, 2006. (available at: https://www.idf.org/e-library/consensus-statements/60-idfconsensus-worldwide-definitionof-the-metabolic-syndrome)

45 Pham MN, Hawa MI, Pfleger C, Roden M, Schernthaner G, Pozzilli P, Buzzetti R, Scherbaum WA, Seissler J, Kolb H, *et al*. Pro- and anti-inflammatory cytokines in latent autoimmune diabetes in adults, type 1 and type 2 diabetes patients: action LADA 4. *Diabetologia* 2011 **54** 1630–1638. (https://doi.org/10.1007/s00125-011-2088-6)

46 Tanti JF, Ceppo F, Jager J & Berthou F. Implication of inflammatory signaling pathways in obesity-induced insulin resistance. *Frontiers in Endocrinology* 2013 **3** 181. (https://doi.org/10.3389/fendo.2012.00181)

47 Bosch M, Lopez-Bermejo A, Vendrell J, Musri M, Ricart W & Fernandez-Real JM. Circulating IL-18 concentration is associated with insulin sensitivity and glucose tolerance through increased fat-free mass. *Diabetologia* 2005 **48** 1841–1843. (https://doi.org/10.1007/s00125-005-1859-3)

48 Ballak DB, Stienstra R, Tack CJ, Dinarello CA & van Diepen JA. IL-1 family members in the pathogenesis and treatment of metabolic disease: focus on adipose tissue inflammation and insulin resistance. *Cytokine* 2015 **75** 280–290. (https://doi.org/10.1016/j.cyto.2015.05.005)

49 Chiu HK, Tsai EC, Juneja R, Stoever J, Brooks-Worrell B, Goel A & Palmer JP. Equivalent insulin resistance in latent autoimmune diabetes in adults (LADA) and type 2 diabetic patients. *Diabetes Research and Clinical Practice* 2007 **77** 237–244. (https://doi.org/10.1016/j.diabres.2006.12.013)

50 Lohmann T, Kellner K, Verlohren HJ, Krug J, Steindorf J, Scherbaum WA & Seissler J. Titre and combination of ICA and autoantibodies to glutamic acid decarboxylase discriminate two clinically distinct types of latent autoimmune diabetes in adults (LADA). *Diabetologia* 2001 **44** 1005–1010. (https://doi.org/10.1007/s001250100602)

51 Katakami N, Kaneto H, Matsuhisa M, Yoshiuchi K, Kato K, Yamamoto K, Umayahara Y, Kosugi K, Hori M & Yamasaki Y. Serum interleukin-18 levels are increased and closely associated with various soluble adhesion molecule levels in type 1 diabetic patients. *Diabetes Care* 2007 **30** 159–161. (https://doi.org/10.2337/dc06-1768)

52 Boraschi D & Dinarello CA. IL-18 in autoimmunity: review. *European Cytokine Network* 2006 **17** 224–252. (https://doi.org/10.1684/ecn.2006.0047)

53 Harms RZ, Yarde DN, Guinn Z, Lorenzo-Arteaga KM, Corley KP, Cabrera MS & Sarvetnick NE. Increased expression of IL-18 in the serum and islets of type 1 diabetics. *Molecular Immunology* 2015 **64** 306–312. (https://doi.org/10.1016/j.molimm.2014.12.012)

54 Zaharieva ET, Velikova TV, Tsakova AD & Kamenov ZA. Prevalence of positive diabetes-associated autoantibodies among type 2 diabetes and related metabolic and inflammatory differences in a sample of the Bulgarian population. *Journal of Diabetes Research* 2017 **2017** 9016148. (https://doi.org/10.1155/2017/9016148)

55 Altinova AE, Yetkin I, Akbay E, Bukan N & Arslan M. Serum IL-18 levels in patients with type 1 diabetes: relations to metabolic control and microvascular complications. *Cytokine* 2008 **42** 217–221. (https://doi.org/10.1016/j.cyto.2008.02.006)

56 Böni-Schnetzler M, Ehses JA, Faulenbach M & Donath MY. Insulitis in type 2 diabetes. *Diabetes, Obesity and Metabolism* 2008 **10** 201–204. (https://doi.org/10.1111/j.1463-1326.2008.00950.x)

57 Maedler K, Sergeev P, Ris F, Oberholzer J, Joller-Jemelka HI, Spinas GA, Kaiser N, Halban PA & Donath MY. Glucose-induced β cell production of IL-1β contributes to glucotoxicity in human pancreatic islets. *Journal of Clinical Investigation* 2002 **110** 851–860. (https://doi.org/10.1172/JCI15318)

58 Hawa MI, Kolb H, Schloot N, Beyan H, Paschou SA, Buzzetti R, Mauricio D, De Leiva A, Yderstraede K, Beck-Neilsen H, *et al*. Adult-onset autoimmune diabetes in Europe is prevalent with a broad clinical phenotype: action LADA 7. *Diabetes Care* 2013 **36** 908–913. (https://doi.org/10.2337/dc12-0931)

59 Lohmann T, Kellner K, Verlohren HJ, Krug J, Steindorf J, Scherbaum WA & Seissler J. Titre and combination of ICA and autoantibodies to glutamic acid decarboxylase discriminate two clinically distinct types of latent autoimmune diabetes in adults (LADA). *Diabetologia* 2001 **44** 1005–1010. (https://doi.org/10.1007/s001250100602)

60 Rolandsson O & Palmer JP. Latent autoimmune diabetes in adults (LADA) is dead: long live autoimmune diabetes! *Diabetologia* 2010 **53** 1250–1253.

Received in final form 30 November 2017
Accepted 7 December 2017
Accepted Preprint published online 7 December 2017