Quantikine® ELISA

Mouse CD14 Immunoassay

Catalog Number MC140

For the quantitative determination of mouse CD14 concentrations in cell culture supernates, serum, and plasma.

This package insert must be read in its entirety before using this product.
For research use only. Not for use in diagnostic procedures.

TABLE OF CONTENTS

SECTION PAGE

INTRODUCTION 1
PRINCIPLE OF THE ASSAY 2
LIMITATIONS OF THE PROCEDURE 2
TECHNICAL HINTS 2
MATERIALS PROVIDED & STORAGE CONDITIONS 3
OTHER SUPPLIES REQUIRED 4
PRECAUTIONS 4
SAMPLE COLLECTION & STORAGE 4
SAMPLE PREPARATION 4
REAGENT PREPARATION 5
ASSAY PROCEDURE 6
CALCULATION OF RESULTS 7
TYPICAL DATA 7
PRECISION 8
RECOVERY 8
LINEARITY 8
SENSITIVITY 9
CALIBRATION 9
SAMPLE VALUES 9
SPECIFICITY 9
REFERENCES 10

MANUFACTURED AND DISTRIBUTED BY:

USA & Canada | R&D Systems, Inc.
614 McKinley Place NE, Minneapolis, MN 55413, USA
TEL: (800) 343-7475 (612) 379-2956 FAX: (612) 656-4400
E-MAIL: info@RnDSystems.com

DISTRIBUTED BY:

UK & Europe | R&D Systems Europe, Ltd.
19 Barton Lane, Abingdon Science Park, Abingdon OX14 3NB, UK
TEL: +44 (0)1235 529449 FAX: +44 (0)1235 533420
E-MAIL: info@RnDSystems.co.uk

China | R&D Systems China Co., Ltd.
24A1 Hua Min Empire Plaza, 726 West Yan An Road, Shanghai PRC 200050
TEL: +86 (21) 52380373 FAX: +86 (21) 52371001
E-MAIL: info@RnDSystemsChina.com.cn

INTRODUCTION

CD14 is an acute phase glycoprotein that binds lipopolysaccharide (LPS) endotoxins with cells, thereby signaling the presence of gram-negative bacteria (1-4). Its 11 leucine-rich repeats mediate the interaction with LPS. The 55 kDa form, mCD14, is anchored to the membrane via glycosylphosphatidylinositol (GPI) linkage (1, 2, 5-7). Human mCD14 shares 63-73% amino acid sequence identity with mouse, rat or rabbit CD14. Soluble forms of CD14, or sCD14, may be secreted prior to GPI linkage or shed by proteolysis or cleavage of the GPI linkage. This variation, plus variable glycosylation, creates forms that may range from 43 to 53 kDa (8-10). In humans, the N terminal 13 kDa, termed presepsin or sCD14-ST, is a small soluble subtype that is found in plasma during sepsis or local infection (11-13). Membrane CD14 is expressed primarily on the cells that are most sensitive to LPS, including monocytes, macrophages and neutrophils (1-3). Lower amounts are detected on other cells, such as B cells, epithelial cells, endothelial cells, and fibroblasts (1, 14-16). Soluble CD14 is found in serum, urine and other body fluids (5).

CD14 cooperates with another acute phase protein, LBP (LPS-binding protein), which binds LPS and transfers it to CD14 (1-3, 17). LPS can be further transferred from CD14 to the TLR4/MD2 complex on the cell surface (1). LPS causes signaling via clustering of LPS-bound CD14/TLR4/MD2 complexes within cholesterol-rich lipid rafts (1). These signals induce production of inflammatory cytokines and other inflammatory proteins (18, 19). When uncoordinated, the signals contribute to septic shock (1). CD14 potentiates TLR-mediated signals triggered by microbe-derived ligands other than LPS, including TLR2 activation by polymeric peptidoglycan, gram-positive bacterial lipoteichoic acid, or mycobacterial lipoarabinomannan (1, 20). It increases uptake and trafficking of the viral TLR3 ligand, poly(I:C) (1, 20). In the lungs, CD14 binds phosphoinositides and surfactant proteins, such as SP-A and SP-D, via interaction of lipids with the CD14 LPS binding site (21, 22). CD14 may also bind ICAM-3 on apoptotic leukocytes and induce phagocytosis (23).

High concentrations of sCD14 may inhibit LPS-mediated responses by competing for binding to mCD14 (24). In the presence of LBP, however, sCD14 can also potentiate LPS responses or help cells to clear circulating LPS, whether or not the cells express mCD14 (16-19). At low concentrations of LPS, both sCD14 and mCD14 can mediate endothelial cell responses such as upregulation of E-selectin, while response to intermediate concentrations of LPS requires mCD14 (16). Circulating sCD14, especially presepsin, is increased in sepsis and may correlate with severity (11, 12). sCD14 may be increased as compared to normal circulating sCD14 in some autoimmune diseases, such as multiple sclerosis and rheumatoid arthritis, but decreased in others, such as Crohn's disease (25-27). sCD14 can also modulate inflammation-driven insulin resistance (28).

The Quantikine® Mouse CD14 Immunoassay is a 4.5 hour solid phase ELISA designed to measure mouse CD14 levels in cell culture supernates, serum, and plasma. It contains NS0-expressed recombinant mouse CD14 and antibodies raised against the recombinant factor. This immunoassay has been shown to quantitate the recombinant mouse CD14 accurately. Results obtained using natural mouse CD14 showed dose-response curves that were parallel to the standard curves obtained using the Quantikine® kit standards. These results indicate that this kit can be used to determine relative mass values for natural mouse CD14.

PRINCIPLE OF THE ASSAY

This assay employs the quantitative sandwich enzyme immunoassay technique. A monoclonal antibody specific for mouse CD14 has been pre-coated onto a microplate. Standards, control, and samples are pipetted into the wells and any CD14 present is bound by the immobilized antibody. After washing away any unbound substances, an enzyme-linked polyclonal antibody specific for mouse CD14 is added to the wells. Following a wash to remove any unbound antibody-enzyme reagent, a substrate solution is added to the wells. The enzyme reaction yields a blue product that turns yellow when the Stop Solution is added. The intensity of the color measured is in proportion to the amount of CD14 bound in the initial step. The sample values are then read off the standard curve.

LIMITATIONS OF THE PROCEDURE

- FOR RESEARCH USE ONLY. NOT FOR USE IN DIAGNOSTIC PROCEDURES.
- The kit should not be used beyond the expiration date on the kit label.
- Do not mix or substitute reagents with those from other lots or sources.
- Samples must be pipetted within 15 minutes.
- If samples generate values higher than the highest standard, further dilute the samples with calibrator diluent and repeat the assay.
- Any variation in diluent, operator, pipetting technique, washing technique, incubation time or temperature, and kit age can cause variation in binding.
- Variations in sample collection, processing, and storage may cause sample value differences.
- This assay is designed to eliminate interference by other factors present in biological samples. Until all factors have been tested in the Quantikine® Immunoassay, the possibility of interference cannot be excluded.

TECHNICAL HINTS

- When mixing or reconstituting protein solutions, always avoid foaming.
- To avoid cross-contamination, change pipette tips between additions of each standard level, between sample additions, and between reagent additions. Also, use separate reservoirs for each reagent.
- To ensure accurate results, proper adhesion of plate sealers during incubation steps is necessary.
- Substrate Solution should remain colorless until added to the plate. Keep Substrate Solution protected from light. Substrate Solution should change from colorless to gradations of blue.
- Stop Solution should be added to the plate in the same order as the Substrate Solution. The color developed in the wells will turn from blue to yellow upon addition of the Stop Solution.

MATERIALS PROVIDED & STORAGE CONDITIONS

Store the unopened kit at 2-8 °C. Do not use past kit expiration date.

PART	PART #	DESCRIPTION	STORAGE OF OPENED/ RECONSTITUTED MATERIAL
Mouse CD14 Microplate	894340	96 well polystyrene microplate (12 strips of 8 wells) coated with a monoclonal antibody specific for mouse CD14.	Return unused wells to the foil pouch containing the desiccant pack. Reseal along entire edge of the zip-seal. May be stored for up to 1 month at 2-8 °C.*
Mouse CD14 Control	894343	2 vials of recombinant mouse CD14 in a buffered protein base with preservatives; lyophilized. The assay value of the control should be within the range specified on the label.	Prepare fresh for each use. Discard after use.
Mouse CD14 Conjugate	894341	12 mL of a polyclonal antibody specific for mouse CD14 conjugated to horseradish peroxidase with preservatives.	May be stored for up to 1 month at 2-8 °C.*
Mouse CD14 Standard	894342	Recombinant mouse CD14 in a buffered protein base with preservatives; lyophilized. *Refer to the vial label for reconstitution volume.*	
Assay Diluent RD1-114	895985	11 mL of a buffered protein base with preservatives.	
Calibrator Diluent RD6-31	895323	2 vials (21 mL/vial) of diluted animal serum with preservatives.	
Wash Buffer Concentrate	895003	21 mL of a 25-fold concentrated solution of buffered surfactant with preservative. *May turn yellow over time.*	
Color Reagent A	895000	12 mL of stabilized hydrogen peroxide.	
Color Reagent B	895001	12 mL of stabilized chromogen (tetramethylbenzidine).	
Stop Solution	895174	23 mL of diluted hydrochloric acid.	
Plate Sealers	N/A	4 adhesive strips.	

* Provided this is within the expiration date of the kit.

OTHER SUPPLIES REQUIRED

- Microplate reader capable of measuring absorbance at 450 nm, with the correction wavelength set at 540 nm or 570 nm.
- Pipettes and pipette tips.
- Deionized or distilled water.
- Squirt bottle, manifold dispenser, or automated microplate washer.
- 500 mL graduated cylinder.
- Horizontal orbital microplate shaker (0.12" orbit) capable of maintaining a speed of 500 ± 50 rpm.
- Test tubes for dilution of standards and samples.

PRECAUTIONS

The Stop Solution provided with this kit is an acid solution.

Some components in this kit contain a preservative which may cause an allergic skin reaction. Avoid breathing mist.

Color Reagent B may cause skin, eye, and respiratory irritation. Avoid breathing fumes.

Wear protective gloves, clothing, eye, and face protection. Wash hands thoroughly after handling. Refer to the MSDS on our website prior to use.

SAMPLE COLLECTION & STORAGE

The sample collection and storage conditions listed below are intended as general guidelines. Sample stability has not been evaluated.

Cell Culture Supernates - Remove particulates by centrifugation. Assay immediately or aliquot and store samples at ≤ -20 °C. Avoid repeated freeze-thaw cycles.

Serum - Allow blood samples to clot for 2 hours at room temperature before centrifuging for 20 minutes at 2000 x g. Remove serum and assay immediately or aliquot and store samples at ≤ -20 °C. Avoid repeated freeze-thaw cycles.

Plasma - Collect plasma using EDTA or heparin as an anticoagulant. Centrifuge for 20 minutes at 2000 x g within 30 minutes of collection. Assay immediately or aliquot and store samples at ≤ -20 °C. Avoid repeated freeze-thaw cycles.

Note: *Citrate plasma has not been validated for use in this assay.*
Grossly hemolyzed samples are not suitable for use in this assay.

SAMPLE PREPARATION

Cell culture supernate samples require at least a 2-fold dilution. A suggested 2-fold dilution is 75 µL of sample + 75 µL of Calibrator Diluent RD6-31.

Serum and plasma samples require a 40-fold dilution. A suggested 40-fold dilution is 10 µL of sample + 390 µL of Calibrator Diluent RD6-31.

REAGENT PREPARATION

Bring all reagents to room temperature before use.

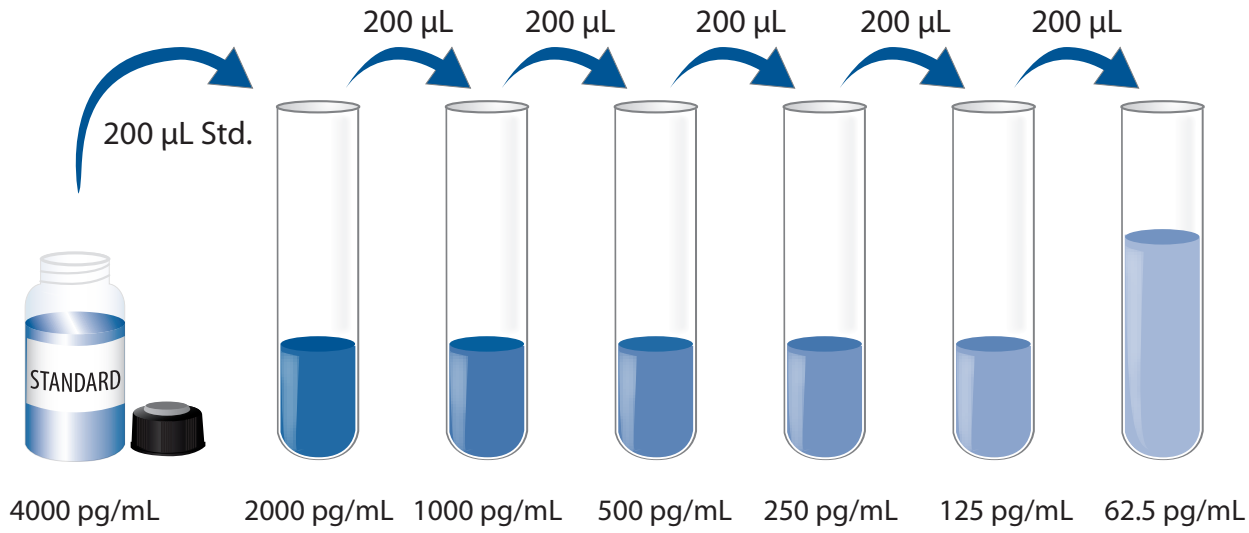
Mouse CD14 Control - Reconstitute the control with 1.0 mL of deionized or distilled water. Mix thoroughly. Assay the control undiluted.

Wash Buffer - If crystals have formed in the concentrate, warm to room temperature and mix gently until the crystals have completely dissolved. Add 20 mL of Wash Buffer Concentrate to deionized or distilled water to prepare 500 mL of Wash Buffer.

Substrate Solution - Color Reagents A and B should be mixed together in equal volumes within 15 minutes of use. Protect from light. 100 µL of the resultant mixture is required per well.

Mouse CD14 Standard - **Refer to the vial label for reconstitution volume.** Reconstitute the Mouse CD14 Standard with Calibrator Diluent RD6-31. This reconstitution produces a stock solution of 4000 pg/mL. Allow the standard to sit for a minimum of 5 minutes with gentle mixing prior to making dilutions.

Pipette 200 µL of Calibrator Diluent RD6-31 into each tube. Use the stock solution to produce a dilution series (below). Mix each tube thoroughly before the next transfer. The undiluted Mouse CD14 Standard (4000 pg/mL) serves as the high standard. Calibrator Diluent RD6-31 serves as the zero standard (0 pg/mL).



ASSAY PROCEDURE

Bring all reagents and samples to room temperature before use. It is recommended that all standards, control, and samples be assayed in duplicate.

1. Prepare all reagents, working standards, control, and samples as directed in the previous sections.
2. Remove excess microplate strips from the plate frame, return them to the foil pouch containing the desiccant pack, and reseal.
3. Add 100 µL of Assay Diluent RD1-114 to each well.
4. Add 50 µL of standard, control, or sample* per well. Cover with the adhesive strip provided. Incubate for 2 hours at room temperature on a horizontal orbital microplate shaker (0.12" orbit) set at 500 ± 50 rpm.

   Note: *Pipette standard, control, and samples within 15 minutes.*
5. Aspirate each well and wash, repeating the process three times for a total of four washes. Wash by filling each well with Wash Buffer (400 µL) using a squirt bottle, manifold dispenser, or autowasher. Complete removal of liquid at each step is essential to good performance. After the last wash, remove any remaining Wash Buffer by aspirating or decanting. Invert the plate and blot it against clean paper towels.
6. Add 100 µL of Mouse CD14 Conjugate to each well. Cover with a new adhesive strip. Incubate for 2 hours at room temperature on the shaker.
7. Repeat the aspiration/wash as in step 5.
8. Add 100 µL of Substrate Solution to each well. Incubate for 30 minutes at room temperature **on the benchtop. Protect from light.**
9. Add 100 µL of Stop Solution to each well. Gently tap the plate to ensure thorough mixing.
10. Determine the optical density of each well within 30 minutes, using a microplate reader set to 450 nm. If wavelength correction is available, set to 540 nm or 570 nm. If wavelength correction is not available, subtract readings at 540 nm or 570 nm from the readings at 450 nm. This subtraction will correct for optical imperfections in the plate. Readings made directly at 450 nm without correction may be higher and less accurate.

*Samples require dilution. See Sample Preparation section.

CALCULATION OF RESULTS

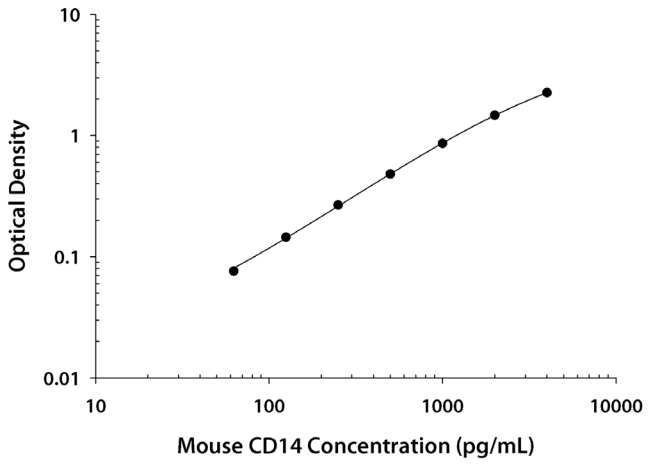
Average the duplicate readings for each standard, control, and sample and subtract the average zero standard optical density (O.D.).

Create a standard curve by reducing the data using computer software capable of generating a four parameter logistic (4-PL) curve-fit. As an alternative, construct a standard curve by plotting the mean absorbance for each standard on the y-axis against the concentration on the x-axis and draw a best fit curve through the points on the graph. The data may be linearized by plotting the log of the mouse CD14 concentrations versus the log of the O.D. and the best fit line can be determined by regression analysis. This procedure will produce an adequate but less precise fit of the data.

Since samples have been diluted, the concentration read from the standard curve must be multiplied by the dilution factor.

TYPICAL DATA

This standard curve is provided for demonstration only. A standard curve should be generated for each set of samples assayed.



(pg/mL)	O.D.	Average	Corrected
0	0.008 0.009	0.009	—
62.5	0.084 0.085	0.085	0.076
125	0.148 0.159	0.154	0.145
250	0.268 0.284	0.276	0.267
500	0.485 0.494	0.490	0.481
1000	0.860 0.879	0.870	0.861
2000	1.453 1.502	1.478	1.469
4000	2.242 2.284	2.263	2.254

PRECISION

Intra-assay Precision (Precision within an assay)
Three samples of known concentration were tested twenty times on one plate to assess intra-assay precision.

Inter-assay Precision (Precision between assays)
Three samples of known concentration were tested in twenty separate assays to assess inter-assay precision. Assays were performed by at least three technicians using two lots of components.

	Intra-Assay Precision			Inter-Assay Precision		
Sample	1	2	3	1	2	3
n	20	20	20	20	20	20
Mean (pg/mL)	177	486	1425	158	433	1416
Standard deviation	6.3	13.7	85.5	13.9	28.2	95.0
CV (%)	3.6	2.8	6.0	8.8	6.5	6.7

RECOVERY

The recovery of mouse CD14 spiked to three levels throughout the range of the assay was evaluated. Samples were diluted prior to assay.

Sample Type	Average % Recovery	Range
Cell culture supernates (n=4)	97	87-107%

LINEARITY

To assess the linearity of the assay, samples containing high concentrations of mouse CD14 were serially diluted with calibrator diluent to produce samples with values within the dynamic range of the assay. Samples were diluted prior to assay.

		Cell culture supernates (n=4)	Serum (n=4)	EDTA plasma (n=4)	Heparin plasma (n=4)
1:2	Average % of Expected	93	105	105	104
	Range (%)	85-97	91-111	100-111	91-111
1:4	Average % of Expected	91	105	106	108
	Range (%)	85-94	97-110	103-108	91-116
1:8	Average % of Expected	93	112	105	110
	Range (%)	84-99	105-120	95-110	100-118
1:16	Average % of Expected	90	108	108	111
	Range (%)	83-101	100-114	100-113	103-118

SENSITIVITY

Twenty-seven assays were evaluated and the minimum detectable dose (MDD) of mouse CD14 ranged from 0.66-6.15 pg/mL. The mean MDD was 2.27 pg/mL.

The MDD was determined by adding two standard deviations to the mean O.D. value of twenty zero standard replicates and calculating the corresponding concentration.

CALIBRATION

This immunoassay is calibrated against a highly purified NS0-derived recombinant mouse CD14 produced at R&D Systems®.

SAMPLE VALUES

Serum/Plasma - Samples were evaluated for the presence of mouse CD14 in this assay.

Sample Type	Mean (ng/mL)	Range (ng/mL)	Standard Deviation (ng/mL)
Serum (n=20)	54.4	34.4-81.0	10.7
EDTA Plasma (n=10)	50.3	34.2-85.8	15.7
Heparin plasma (n=10)	50.9	36.2-71.1	12.0

Cell Culture Supernates - IC-21 mouse macrophages (5 x 10^4 cells/mL) were cultured in RPMI supplemented with 10% fetal bovine serum, 2 mM L-glutamine, 100 U/mL penicillin, and 100 µg/mL streptomycin sulfate. Cells were unstimulated or stimulated with 5.0 µg/mL of LPS and 100 ng/mL of recombinant mouse IL-10 (R&D Systems®, Catalog # 417-ML) for 3 days. Aliquots of the cell culture supernates were removed, assayed for mouse CD14, and measured 1015 pg/mL and 1784 pg/mL, respectively.

SPECIFICITY

This assay recognizes natural and recombinant mouse CD14.

The factors listed below were prepared at 50 ng/mL in calibrator diluent and assayed for cross-reactivity. Preparations of the following factors at 50 ng/mL in a mid-range recombinant mouse CD14 control were assayed for interference. No significant cross-reactivity or interference was observed.

Recombinant mouse:

TLR-2

Recombinant human:

ICAM-3
LBP
MD-2
TLR-4
TLR-4/MD-2 Complex

Recombinant human CD14 cross-reacts approximately 0.19% in this assay.

Lipopolysaccharide interferes at levels > 1.0 µg/mL.

REFERENCES

1. Anas, A. *et al.* (2010) Crit. Care **14**:209.
2. Wright, S.D. *et al.* (1990) Science **249**:1431.
3. Schumann, R.R. (1992) Res. Immunol. **143**:11.
4. Aalto, H. *et al.* (2007) Scand. J. Infect. Dis. **39**:596.
5. Bazil, V. *et al.* (1989) Mol. Immunol. **26**:657.
6. Simmons, D.L. *et al.* (1989) Blood **73**:284.
7. Haziot, A. *et al.* (1988) J. Immunol. **141**:547.
8. Durieux, J.J. *et al.* (1994) Eur. J. Immunol. **24**:2006.
9. Bufler, P. *et al.* (1995) Eur. J. Immunol. **25**:604.
10. Stelter, F. *et al.* (1996) Eur. J. Biochem. **236**:457.
11. Yaegashi, Y. *et al.* (2005) J. Infect. Chemother. **11**:234.
12. Shozushima, T. *et al.* (2011) J. Infect. Chemother. **17**:764.
13. Shirakawa, K. *et al.* (2011) Clin. Chem. Lab. Med. **49**:937.
14. Ziegler-Heitbrock, H.W.L. *et al.* (1994) Eur. J. Immunol. **24**:1937.
15. Ferrero, E. *et al.* (1998) J. Immunol. **160**:2675.
16. Lloyd-Jones, K.L. *et al.* (2008) J. Immunol. **181**:1446.
17. Tapping, R.I. and P.S. Tobias (1997) J. Biol. Chem. **272**:23157.
18. Frey, E.A. *et al.* (1992) J. Exp. Med. **176**:1665.
19. Pugin, J. *et al.* (1993) Proc. Natl. Acad. Sci. USA **90**:2744.
20. Weidemann, B. *et al.* (1997) Infect. Immun. **65**:858.
21. Kuronuma, K. *et al.* (2009) J. Biol. Chem. **284**:25488.
22. Wang, P.Y. *et al.* (1998) J. Biol. Chem. **273**:24309.
23. Moffatt, O.D. *et al.* (1999) J. Immunol. **16**2:6800.
24. Grunwald, U. *et al.* (1993) Circul. Shock **39**:220.
25. Lakatos, P.L. *et al.* (2011) Inflamm. Bowel. Dis. **17**:767.
26. Lutterotti, A. *et al.* (2006) J. Neuroimmunol. **181**:145.
27. Mikuls, T.R. *et al.* (2011) J. Rheumatol. **38**:2509.
28. Fernandez-Real, J.M. *et al.* (2011) Diabetes **60**:2179.