

**Citation:**

Monagas M, Khan N, Andres-Lacueva C, Casas R, Urpí-Sardà M, Llorach R, Lamuela-Raventós RM, Estruch R. Effect of cocoa powder on the modulation of inflammatory biomarkers in patients at high risk of cardiovascular disease. *Am J Clin Nutr*. 2009 Sep 23. [Epub ahead of print]

**PubMed ID:** [19776136](#)

**Study Design:**

Randomized Crossover Trial

**Class:**

A - [Click here](#) for explanation of classification scheme.

**Research Design and Implementation Rating:**

 NEUTRAL: See Research Design and Implementation Criteria Checklist below.

**Research Purpose:**

The objective was to evaluate the effects of chronic cocoa consumption on cellular and serum biomarkers related to atherosclerosis in high-risk patients.

**Inclusion Criteria:**

- Recruited from the outpatient clinic of the Internal Medicine Department of University of Barcelona, Barcelona, Spain
- $\geq 55$  years
- Had diabetes mellitus
- Had  $\geq 3$  of the following cardiovascular risk factors: tobacco smoking, hypertension, plasma LDL cholesterol  $\geq 160$  mg/dl, plasma HDL cholesterol  $\leq 35$  mg/dl, obesity (body mass index)  $\geq 30$ , and/or family history of premature CHD

**Exclusion Criteria:**

- Documented CHD
- Stroke or peripheral arteriopathy
- History of allergic reactions to any cocoa components

**Description of Study Protocol:****Recruitment**

High risk subjects were recruited for the study in the outpatient clinic of the Internal Medicine Department.

**Design**

The study was designed as a randomized, crossover, controlled clinical trial consisting of two 4-week periods.

### **Blinding used (if applicable)**

The clinical investigators and laboratory technicians were blinded to the interventions.

### **Intervention (if applicable)**

- All participants followed an isocaloric Mediterranean-type diet
- C+M intervention: 40 g cocoa + 500 ml skim milk
- M intervention: 500 ml skim milk

### **Statistical Analysis**

- Statistical analysis was performed by using the SPSS Statistical Analysis System (version 14.0, SPSS, Inc, Chicago, IL).
- Descriptive statistics (mean  $\pm$  SD) were used for the baseline characteristics of the participants.
- For analysis of laboratory variables, the average of the 2 measures taken after each intervention was used in the comparison between the 2 interventions.
- Values with a skewed distribution (CRP, VCAM-1, ICAM-1, and IL-6) were transformed to their natural logarithm for analysis.
- One-factor analysis of variance (ANOVA) for repeated measures with the Bonferroni post hoc test, adjusted by age and sex, was used to compare changes in outcome variables in response to the intervention treatments.
- Within- and between-group differences are expressed as means and 95% CIs.
- *P* was considered significant when  $<0.05$ .

### **Data Collection Summary:**

#### **Timing of Measurements**

Baseline and before and after each 4 week intervention period.

#### **Dependent Variables**

- Anthropometric and blood pressure measurements: performed with standardized methods
- Measured in fasting blood samples at baseline and after each intervention: blood glucose, cholesterol, triglycerides, HDL cholesterol, hs-CRP, soluble intercellular adhesion molecule-1 (ICAM-1), vascular cell adhesion molecule (VCAM-1), E-selectin, P-selectin, and monocyte chemoattractant protein (MCP-1) and interleukin-6 (IL-6)
- Measured from 24-hour urine specimen: epicatechin metabolites derived from phase II metabolism

#### **Independent Variables**

- C+M Intervention: 40 g cocoa powder with 500 ml skim milk
- M Intervention: 500 ml skim milk

#### **Control Variables**

### **Description of Actual Data Sample:**

**Initial N:** 47 eligible subjects

**Attrition (final N):** 42 volunteers included in the study (19 men and 23 women)

**Age:** mean  $69.7 \pm 11.5$  years

**Ethnicity:** not reported

**Other relevant demographics and Anthropometrics:** estimated mean (95% CI)

Weight	73.6 (69.6,77.7)
BMI (kg/m <sup>2</sup> )	27.6 (26.0,29.1)
Systolic blood pressure (mmHg)	138 (130,146)
Diastolic blood pressure (mmHg)	84 (80,88)
Heart rate (beats/min)	73 (67,78)
Glucose (mg/dL)	121 (109,133)
Total cholesterol (mg/dL)	225 (212,238)
Triglycerides (mg/dL)	127 (106,146)
LDL cholesterol (mg/dL)	177 (165,188)
HDL cholesterol (mg/dL)	51.8 (47.7,55.9)

**Location:**Barcelona, Spain

**Summary of Results:**

**Key Findings**

- No significant changes in the expression of adhesion molecules on T lymphocyte surfaces were found between the C+M and M groups.
- In monocytes, the expression of VLA4, CD40, and CD35 was significantly lower ( $P = 0.005, 0.028,$  and  $0.001,$  respectively) after C+M intake than after M intake.
- Serum concentrations of the soluble endothelium-derived adhesion molecules P-selectin and intercellular adhesion molecule-2 were significantly lower (both  $P = 0.007$ ) after C+M intake than after M intake.

**Expression of adhesion molecules on the surface of T lymphocytes and monocytes in 42 subject studied at baseline and after both interventions**

	Baseline	C+M intervention	M intervention	<i>P</i>
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T lymphocytes (MFI)				
LFA-1	73.03(65.70,78.57)	76.34(71.93,80.75)	78.72(74.86,82.58)	0.380
VLA-4	53.42(52.44,54.30)	54.07(53.24,54.89)	53.96(53.05,54.87)	0.359
SLe	127.83(111.54,138.12)	125.99(111.23,140.75)	133.87(123.82,143.92)	0.361
CD40	60.46(54.23,66.70)	57.70(53.36,62.05)	56.03(52.02,60.04)	0.461
Monocytes (MFI)				
LFA-1	32.20(29.96,34.44)	30.83(28.96,32.69)	30.01(28.27,31.76)	0.310
Mac-1	33.65(30.56,34.44)	35.76(32.97,38.54)	35.11(32.93,37.29)	0.405
VLA-4	25.17(23.92,26.42)	22.96(22.14,23.78)	24.79(23.85,25.74)	0.039
SLe <sup>x</sup>	57.53(52.43,62.63)	60.70(54.69,66.71)	61.29(55.92,66.65)	0.420
CD40	24.53(23.59,25.47)	<b>23.31(22.52,24.05)</b>	24.95(23.86,26.02)	0.031
CD36	26.01(24.02,28.02)	<b>22.61(20.84,24.57)</b>	28.69(26.60,30.88)	0.038

**Bold** values are significantly different,  $P < 0.05$

#### Circulating inflammatory markers in the 42 subjects studied at baseline and after both interventions

	Baseline	C+M intervention	M intervention	<i>P</i>
Soluble adhesion molecules				
P-selectin (ng/mL)	255.02(203.39,306.49)	<b>253.39(187.03,283.75)</b>	263.87(215.45,312.28)	0.031
E-selectin (ng/mL)	45.41(36.92,53.90)	46.02(36.33,55.72)	45.72(37.55,53.96)	0.211
ICAM-1 (ng/mL)	359.07(316.13,402.00)	<b>331.47(285.30,377.64)</b>	267.23(317.31,417.15)	0.034
VCAM-1 (ng/mL)	992.07(846.55,1137.58)	986.31(850.18,1122.43)	1026.90(856.91,1196.90)	0.349
MCP-1 (ng/mL)	289.99(244.83,355.15)	280.11(255.54,332.68)	294.24(234.76,353.72)	0.239
Proinflammatory cytokines				
IL-6 (pg/mL)	1.02(0.67,1.37)	1.07(0.75,1.39)	1.13(0.74,1.52)	0.253
Other inflammatory markers				
hs-CRP (mg/dL)	0.52(0.30,-0.73)	0.50(0.36,0.65)	0.53(0.34,0.71)	0.726

**Bold** values are significantly different,  $P < 0.05$

#### Author Conclusion:

These results suggest that the intake of cocoa polyphenols may modulate inflammatory mediators in patients at high risk of cardiovascular disease. These antiinflammatory effects may contribute to the overall benefits of cocoa consumption against atherosclerosis.

#### Reviewer Comments:

*All participants were following an isocaloric Mediterranean-type diet. Interventions only lasted 4 weeks; no washout period between interventions.*

#### Research Design and Implementation Criteria Checklist: Primary Research

##### Relevance Questions

- |    |   |     |
|----|---|-----|
| 1. | Would implementing the studied intervention or procedure (if found successful) result in improved outcomes for the patients/clients/population group? (Not Applicable for some epidemiological studies) | Yes |
| 2. | Did the authors study an outcome (dependent variable) or topic that the patients/clients/population group would care about?   | Yes |
| 3. | Is the focus of the intervention or procedure (independent variable) or topic of study a common issue of concern to nutrition or dietetics practice?  | Yes |
| 4. | Is the intervention or procedure feasible? (NA for some epidemiological studies)  | Yes |

##### Validity Questions

- |      |   |     |
|------|---|-----|
| 1.   | <b>Was the research question clearly stated?</b>  | Yes |
| 1.1. | Was (were) the specific intervention(s) or procedure(s) [independent variable(s)] identified?   | Yes |
| 1.2. | Was (were) the outcome(s) [dependent variable(s)] clearly indicated?  | Yes |
| 1.3. | Were the target population and setting specified?   | Yes |
| 2.   | <b>Was the selection of study subjects/patients free from bias?</b>   | Yes |
| 2.1. | Were inclusion/exclusion criteria specified (e.g., risk, point in disease progression, diagnostic or prognosis criteria), and with sufficient detail and without omitting criteria critical to the study? | Yes |
| 2.2. | Were criteria applied equally to all study groups?  | Yes |

2.3.	Were health, demographics, and other characteristics of subjects described?	Yes
2.4.	Were the subjects/patients a representative sample of the relevant population?	Yes
<b>3.</b>	<b>Were study groups comparable?</b>	Yes
3.1.	Was the method of assigning subjects/patients to groups described and unbiased? (Method of randomization identified if RCT)	Yes
3.2.	Were distribution of disease status, prognostic factors, and other factors (e.g., demographics) similar across study groups at baseline?	Yes
3.3.	Were concurrent controls used? (Concurrent preferred over historical controls.)	Yes
3.4.	If cohort study or cross-sectional study, were groups comparable on important confounding factors and/or were preexisting differences accounted for by using appropriate adjustments in statistical analysis?	N/A
3.5.	If case control or cross-sectional study, were potential confounding factors comparable for cases and controls? (If case series or trial with subjects serving as own control, this criterion is not applicable. Criterion may not be applicable in some cross-sectional studies.)	N/A
3.6.	If diagnostic test, was there an independent blind comparison with an appropriate reference standard (e.g., "gold standard")?	N/A
<b>4.</b>	<b>Was method of handling withdrawals described?</b>	Yes
4.1.	Were follow-up methods described and the same for all groups?	Yes
4.2.	Was the number, characteristics of withdrawals (i.e., dropouts, lost to follow up, attrition rate) and/or response rate (cross-sectional studies) described for each group? (Follow up goal for a strong study is 80%.)	Yes
4.3.	Were all enrolled subjects/patients (in the original sample) accounted for?	Yes
4.4.	Were reasons for withdrawals similar across groups?	Yes
4.5.	If diagnostic test, was decision to perform reference test not dependent on results of test under study?	N/A
<b>5.</b>	<b>Was blinding used to prevent introduction of bias?</b>	Yes
5.1.	In intervention study, were subjects, clinicians/practitioners, and investigators blinded to treatment group, as appropriate?	Yes
5.2.	Were data collectors blinded for outcomes assessment? (If outcome is measured using an objective test, such as a lab value, this criterion is assumed to be met.)	Yes
5.3.	In cohort study or cross-sectional study, were measurements of outcomes and risk factors blinded?	N/A
5.4.	In case control study, was case definition explicit and case ascertainment not influenced by exposure status?	N/A
5.5.	In diagnostic study, were test results blinded to patient history and other test results?	N/A

<b>6.</b>	<b>Were intervention/therapeutic regimens/exposure factor or procedure and any comparison(s) described in detail? Were intervening factors described?</b>	???
6.1.	In RCT or other intervention trial, were protocols described for all regimens studied?	Yes
6.2.	In observational study, were interventions, study settings, and clinicians/provider described?	N/A
6.3.	Was the intensity and duration of the intervention or exposure factor sufficient to produce a meaningful effect?	???
6.4.	Was the amount of exposure and, if relevant, subject/patient compliance measured?	Yes
6.5.	Were co-interventions (e.g., ancillary treatments, other therapies) described?	Yes
6.6.	Were extra or unplanned treatments described?	N/A
6.7.	Was the information for 6.4, 6.5, and 6.6 assessed the same way for all groups?	Yes
6.8.	In diagnostic study, were details of test administration and replication sufficient?	N/A
<b>7.</b>	<b>Were outcomes clearly defined and the measurements valid and reliable?</b>	???
7.1.	Were primary and secondary endpoints described and relevant to the question?	Yes
7.2.	Were nutrition measures appropriate to question and outcomes of concern?	Yes
7.3.	Was the period of follow-up long enough for important outcome(s) to occur?	???
7.4.	Were the observations and measurements based on standard, valid, and reliable data collection instruments/tests/procedures?	Yes
7.5.	Was the measurement of effect at an appropriate level of precision?	Yes
7.6.	Were other factors accounted for (measured) that could affect outcomes?	???
7.7.	Were the measurements conducted consistently across groups?	Yes
<b>8.</b>	<b>Was the statistical analysis appropriate for the study design and type of outcome indicators?</b>	Yes
8.1.	Were statistical analyses adequately described and the results reported appropriately?	Yes
8.2.	Were correct statistical tests used and assumptions of test not violated?	Yes
8.3.	Were statistics reported with levels of significance and/or confidence intervals?	Yes
8.4.	Was "intent to treat" analysis of outcomes done (and as appropriate, was there an analysis of outcomes for those maximally exposed or a dose-response analysis)?	Yes
8.5.	Were adequate adjustments made for effects of confounding factors that might have affected the outcomes (e.g., multivariate analyses)?	Yes

8.6.	Was clinical significance as well as statistical significance reported?	Yes
8.7.	If negative findings, was a power calculation reported to address type 2 error?	N/A
<b>9.</b>	<b>Are conclusions supported by results with biases and limitations taken into consideration?</b>	???
9.1.	Is there a discussion of findings?	Yes
9.2.	Are biases and study limitations identified and discussed?	???
<b>10.</b>	<b>Is bias due to study's funding or sponsorship unlikely?</b>	Yes
10.1.	Were sources of funding and investigators' affiliations described?	Yes
10.2.	Was the study free from apparent conflict of interest?	Yes

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