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Suppressed cytokine production in whole blood cultures may be related to iron status and hepcidin and is partially corrected following weight reduction in morbidly obese pre-menopausal women

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Abstract

Objective—Assess *ex vivo* whole-blood (WB) cytokine production and its association with iron status and serum hepcidin in obese *versus* non-obese women. Determine the change in *ex vivo* WB cytokine production six months after restrictive bariatric surgery in the obese group. Subjects: 17 obese (BMI: 46.6 ±7.9 kg/m^2) and 19 non-obese (BMI: 22.5 ± 3.0 kg/m^2), pre-menopausal women; frequency matched for hemoglobin, age and race.

Measurements—At baseline control and *ex vivo* stimulated IL-6, IL-10, IL-22, IFNγ, and TNFα from heparinized WB cultures, hemoglobin from finger-stick and transferrin receptor, hepcidin, CRP, IL-6, HOMA-IR from fasted serum samples and anthropometric parameters were assessed in the women. All parameters were reassessed six-months following restrictive bariatric surgery in the obese women only.

Results—Whole blood *ex vivo* LPS and ZY stimulated production of IL-6, TNFα and IFNγ was reduced, IL-22 increased, and IL-10 was unaffected in obese compared with the non-obese women. Furthermore, *ex vivo* stimulated production of IL-6 and TNFα normalized, but IFNγ

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production remained unchanged with weight loss following restrictive bariatric surgery. In the obese women, serum transferrin receptor (a marker of iron status) and serum hepcidin were correlated with *ex vivo* stimulated IFNγ production at baseline.

Conclusion—*Ex vivo* LPS and ZY stimulated cytokine production from WB cultures was altered in pre-menopausal women with morbid obesity. Significant weight loss resulted in normalization of some but not all observed alterations. Furthermore, iron status and serum hepcidin were associated with *ex vivo* LPS and ZY stimulated IFNγ in obesity.

Keywords

Obesity; *ex vivo* LPS and ZY stimulated cytokine production; immunity; hepcidin; iron status; weight loss

1.0 Introduction

Obesity is accompanied by a state of chronic low-grade inflammation stemming from the expanded adipose tissue mass, which is infiltrated by activated leukocytes that produce cytokines and other inflammatory mediators (1). In particular, elevated systemic levels of the cytokine IL-6 and the acute-phase protein C reactive protein (CRP) are present in obese subjects (2). Although the effect of obesity on immune responses has yet to be fully characterized, available data indicate impaired function of Natural Killer (NK) cells and macrophages in obese humans and mice (3,4) as well as potential deleterious effects of obesity on T lymphocyte maturation (5). Other reports show that obesity biases lymphocytes toward a pro-inflammatory T helper (Th) 17 phenotype in mice as well as the presence of hyper-responsive neutrophils (6–8). Thus, the presence of chronic inflammation in adipose tissue of obese individuals may be associated with both impaired systemic immune responses and a hyper-activated inflammatory state.

Iron is a critical element in regulation of the immune response and is integral to the proliferation of T, B, and NK cells (9,10). Th1-mediated immune function is thought to be more sensitive to iron homeostasis *in vivo* compared to the Th2 subset (11). Specifically, iron has a direct inhibitory effect on production and activity of IFNγ, including induction of nitric oxide, an important mediator used by macrophages in fighting pathogens (11,12). Iron depletion has been associated with both immune enhancement, when coupled with malaria in non-obese children (13–16), and immune impairment in non-inflamed normal-weight populations (17,18). Our group and others have reported that obesity in pre-menopausal women is associated with both iron depletion and increased inflammation (19–21). Any benefit or disadvantage of iron depletion on immune function in obese populations is likely limited.

Hepcidin is an anti-microbial peptide involved in innate immunity and is the body's main regulator of systemic iron homeostasis (22,23). Increased expression of hepcidin occurs with inflammation and elevated iron stores, whereas hepcidin expression decreases with hypoxia and erythropoiesis. Recently we demonstrated that obese women have elevated serum hepcidin levels, despite iron depletion, compared to non-obese women with similar hemoglobin (Hb) concentrations, suggesting that hepcidin production in obesity is regulated by the presence of chronic low-grade inflammation rather than by low iron status (21,24). The relationship between iron status, serum hepcidin and immunity in obese populations has yet to be established.

The primary aim of this study was to assess differences in *ex vivo* whole-blood (WB) cytokine production in obese *versus* non-obese women and change six-months following restrictive bariatric surgery in the obese group. Whole-blood was stimulated with agonists of

toll-like receptor (TLR)-4 (LPS) and TLR-2 (Zymosan) that have been implicated in adipose tissue inflammation in obesity (25). The cytokines IL-6, IL-10, IL-22, IFNγ and TNFα were selected for measurement based on their association with obesity and/or iron status (11,26–29). Secondly, we explored relationships between iron status, serum hepcidin, and anthropometrics measures with *ex vivo* WB cytokine production in the obese and non-obese women at baseline and in the obese women post-surgery. We hypothesized that: 1) obese women would have higher *ex-vivo* cytokine production compared to non-obese women, 2) weight loss would result in reduced *ex- vivo* cytokine production in the obese women, and 3) iron sufficiency in the obese women would be associated with increased *ex-vivo* cytokine production.

Methods

2.1 Subjects

Participants in this study were a sub-sample of a larger study of obese and non-obese pre-menopausal women in which the relationship between obesity, iron status, hepcidin and inflammation was investigated before and in the obese six-months following restrictive bariatric surgery. For this ancillary study, participants included obese women evaluated at baseline and at six months post-restrictive gastric surgery (n=17) and non-obese women (n=19) frequency matched for race/ethnicity, age, and Hb levels that consented to providing an additional vial of blood for analysis of WB cytokine production. Detailed methods, procedures and findings from the parent study have been described elsewhere (21,24). Briefly, obese women [body mass index (BMI) > 37.0 kg/m2] electing to undergo a restrictive bariatric procedure (gastric banding or sleeve gastrectomy) were recruited from the University of Illinois at Chicago bariatric surgery clinics between December 2007 and July 2008. As a comparison group, non-obese women (BMI < 27.0 kg/m2, waist circumference < 88.0 cm), free of inflammation, and matched to the obese for Hb, were recruited. A total of 45 non-obese women were screened in order to frequency match to the obese woman for Hb, age, and race/ethnicity. Restricting the comparison group to non-obese women with similar Hb allowed us to determine if hepcidin in obese individuals was responding appropriately to their sub-optimal iron status or if inflammation was counter-regulating this response; additional detail regarding the reasoning behind frequency matching on Hb is available in Tussing-Humphreys et al., 2010 (21). Women were excluded if they reported significant medical conditions that could influence iron or inflammatory status. Dietary iron supplements, vitamins containing iron or non-steroidal anti-inflammatory drugs (NSAIDS) were not consumed 48 hours prior to each research appointment to eliminate the potential acute effects on inflammation or hepcidin production. Subjects that reported a cold, flu or urinary tract infection within the preceding two weeks were rescheduled for a later date. Obese subjects were asked to report for their baseline visit prior to starting a mandatory 10–21 day pre-surgery liquid fast. The research protocol was approved by the University of Illinois at Chicago Institutional Review Board and participants provided written consent prior to study entry.

At baseline and six months post-surgery (obese only), subjects reported after at least an 8 hour fast for a detailed history and clinical examination. Subjects were weighed using a digital scale to the nearest 0.1 kg (Tanita BWB-800AS; Tanita, Arlington Heights, IL), height was measured to the nearest 0.1 cm using a stadiometer and weight circumference was assessed to the nearest 0.1 cm using a flexible tape measure. Demographic, social, reproductive, and health history data were collected via self-report. Usual dietary intake was assessed using a food frequency questionnaire (30) and physical activity was reported using the Kaiser Physical Activity Survey (31). Heparinized WB was obtained and used for evaluation of cytokine production as detailed below.

2.2 Whole-blood Cultures

One milliliter of heparinized WB was diluted with one milliliter of RPMI containing penicillin/streptomycin in the absence or presence of either lipopolysaccharide (LPS, O55:B5 from *E. coli*, Sigma, St. Louis, MO) at 10 ng/ml or zymosan (ZY) (Sigma-Aldrich Co., St. Louis, MO) at 10 μg/ml. Samples were incubated for 48 h at 37°C in a humidified atmosphere with 5% CO_2. The length of incubation was chosen based on previously published experiments (32) and on pilot studies indicating that it is an optimal time point for evaluation of both early, i.e., TNFα and late, i.e., IFNγ. The supernatant was collected and stored at −70°C.

2.3 Cytokine measurement

Levels of IFNγ, TNFα and IL-6 were measured using ELISA kits from R&D Systems (Minneapolis, MN). Levels of IL-10 and IL-22 were measured using ELISA kits from eBioscience (San Diego, CA).

2.4 Additional Biochemical Indices

All biochemical assays were performed on fasted blood samples. Hemoglobin was assessed via finger-stick puncture using a hemoglobinometer (STAT-site Mβ-Hb; Stanbio Laboratories, Boerne, TX). High sensitivity C-reactive protein (CRP), glucose and insulin were performed by Specialty Laboratories (Valencia, CA). The analysis of CRP was by immuno-turbidity (reference interval < 1.0 mg/L), insulin by chemiluminescence (reference range: 3.0–28.0 mU/L), and glucose by hexokinase endpoint spectrophotometry (reference range: 74–106 mg/dL). Insulin resistance was determined by the homeostasis model assessment (HOMA-IR) according to the following formula: [(glucose (mg/dL)/18) X insulin (mU/liter)] /22.5. Serum transferrin receptor (sTfR) was measured by ELISA (R&D Systems). Serum hepcidin was assessed using a competitive ELISA (Intrinsic Life Sciences, La Jolla, CA). Detailed methods and performance of this assay were recently published (33).

2.5 Statistical Analysis

Anthropometric, biochemical, and WB stimulated cytokines are presented as means ± SD. Crude comparisons of WB cytokine stimulations, anthropometrics and biochemical variables at baseline of the obese and non-obese women were assessed using Student's t-test or Wilcoxon rank sum test. In the obese women, WB cytokine stimulations, anthropometrics, and biochemical differences between baseline and six months post-surgery were assessed using paired t-tests or Wilcoxon signed-rank test. To control for multiple comparisons between groups, the statistical significance level was set at $\alpha=0.025$ for these analyses. Additionally, Pearson's and Spearman's bivariate analyses and regression analysis were utilized to test for associations between anthropometric and biochemical measures with LPS and ZY-stimulated WB cytokine production at baseline in both groups. Finally, correlation analysis was performed to assess for relationships between the anthropometric and biochemical measures with LPS and ZY-stimulated WB cytokine production post-surgery and changes in these parameters from baseline in the obese women only. All analyses were performed using SAS (version 9.2, 2002–2008, SAS Institute Inc, Cary, NC).

3.0 Results

3.1 Clinical Characteristics

The baseline and six-month post-surgery clinical characteristics are presented in Table 1. By design, obese and non-obese women had similar Hb and were dissimilar anthropometrically. Both groups of women were comparable for race/ethnicity and age (data not shown). Obese women had significantly higher hs-CRP and HOMA-*IR* compared to the non-obese women.

Despite frequency matching on Hb, obese women had slightly higher sTfR and significantly elevated serum hepcidin compared to the non-obese women. Interleukin-6 was not statistically different between the groups but trended higher in the obese women (p=0.07). Six months post-surgery, anthropometric measures, CRP, IL-6, HOMA-IR, sTfR, and serum hepcidin were significantly lower and Hb significantly increased in the obese women.

3.2 Whole-Blood cytokine production

At baseline, IFNγ levels were significantly lower in WB control cultures from obese women, whereas production of IL-6, IL-10, IL-22 and TNFα in control cultures was comparable between obese and non-obese women (Table 1). Stimulation with LPS and ZY induced significantly lower levels of IL-6, TNF-α and IFNγ in WB cultures from obese compared to the non-obese women. Conversely, LPS and ZY-stimulated IL-22 levels were significantly higher in the obese compared to the non-obese group; IL-10 levels were comparable between the groups under each culture condition.

LPS and ZY stimulated IL-6 and TNFα significantly increased six months post-restrictive gatric surgery compared to baseline in the obese women (Table 1) and differences between non-obese and obese women for these two cytokines became insignificant (data not shown). Minimal improvement in unstimulated, LPS- and ZY-stimulated IFNγ production was observed post-surgery; IFNγ levels remained significantly lower than those reported in the non-obese women. No significant changes in stimulated IL-10 or IL-22 production were noted post-surgery.

3.3 Correlations and linear modeling of WB stimulated cytokines with anthropometric and biochemical variables at baseline

LPS- and ZY-stimulated IFNγ was inversely correlated with hepcidin while ZY-stimulated IFNγ only was positively correlated with sTfR in the obese women (Table 2). Moreover, in multivariable linear modeling, the most parsimonious model predicting log LPS-stimulated IFNγ included only serum hepcidin (β=−6.19; SE=2.02; p=0.008) (data not shown). The most parsimonious model predicting log ZY- stimulated IFNγ included both serum hepcidin (β= −0.01; SE=0.002; p=0.006) and log sTfR (β=−0.07; SE=0.03; p=0.05). No significant relationships between LPS- or ZY-stimulated IFNγ with anthropometric or biochemical variables were identified in the non-obese women. Furthermore, no significant relationships between LPS- or ZY-stimulated TNFα, IL-6, IL-10, or IL-22 with anthropometric or biochemical variables were identified at baseline in either the obese or non-obese women (data not shown).

3.4 Post-surgery Correlations of WB stimulated cytokines with anthropometric and biochemical variables, Obese only

The strong correlations identified at baseline between LPS- and ZY-stimulated IFNγ with serum hepcidin and sTfR weakened but strengthened with Hb and waist circumference six months post-surgery (Table 3). The relationship between six month values for LPS-stimulated TNF-α and serum IL-6 also strengthened post-surgery. Additionally, we assessed the relationship between six month change (Post-surgery – Baseline = Δ) in WB stimulated cytokines with six-month change in anthropometric and biochemical variables (results not shown). Change in body mass was significantly correlated with change in ZY stimulated IL-6 (r=−0.53; p=0.02). Surprisingly, no other biochemical or anthropometric measures were found to be significantly correlated with six-month change in *ex vivo* WB cytokine production in bivariate analysis. However, change in body mass was marginally correlated with change in sTfR (r=0.50; p=0.05) and change in serum hepcidin was associated with change in serum IL-6 (r=0.50; p=0.04), respectively.

4.0 Discussion

In the present report we demonstrated that *ex vivo* LPS and ZY stimulated production of IL-6, TNFα and IFNγ were significantly reduced and IL-22 increased whereas IL-10 unaffected in obese compared with non-obese women. Furthermore, *ex vivo* LPS and ZY stimulated production of IL-6 and TNFα normalized, while IFNγ production remained relatively unchanged with weight loss six month post-restrictive bariatric surgery. Finally, our data indicated that iron status and serum hepcidin were associated with *ex vivo* LPS and ZY stimulated IFNγ production in obesity.

Although our study did not address the mechanisms of altered *ex vivo* WB cytokine production in obesity, this effect is unlikely to be secondary to a generalized hypo-responsiveness of immune cells of obese women to microbial stimuli, even though obesity has been associated with increased risk of infection in both experimental animals and humans (34,35). In fact, whereas production of some cytokines (IL-6, TNFα, and IFNγ) was significantly reduced in WB cultures of obese women, production of IL-10 was unchanged and levels of IL-22 were higher than those in non-obese women. Furthermore, weight loss was associated with normalized production of some cytokines but not others. These data suggest that the link between obesity and cytokine production may be specific for each mediator.

Altered *ex vivo* cytokine production in obesity has been reported by several studies under various culture conditions and types of stimulation, though there is heterogeneity in the mediators affected and the directionality of the change (36–40). Our data indicate that *ex vivo* production of IL-6 and TNFα in response to microbial stimulation is suppressed in obese women. Further, weight loss with the associated reduction in systemic inflammation and amelioration of iron status appears sufficient to normalize production of these two mediators.

We recently reported increased levels of IL-22 in obese mice with acute pancreatitis (29), however, the effect of obesity on IL-22 in humans was not known. Our findings indicate that obese women produce significantly higher levels of IL-22 in response to LPS and ZY compared with non-obese women and that weight loss does not significantly alter *ex vivo* IL-22 production. However, at variance with IL-6, TNFα and IFNγ, no significant correlations were observed between anthropometric and clinical variables and IL-22 production. The mechanisms of regulation of IL-22 production, particularly in the context of inflammation and innate immunity, and the complex role played by this cytokine in various pathophysiological conditions are an area of active investigation (41). Our data indicate that IL-22 production is dysregulated in obesity, a finding that warrants further investigation.

The most consistent relationship between levels of adiposity and *ex vivo* cytokine production has been reported for IFNγ, which is significantly reduced in obesity (36–38) and increased in subjects with anorexia nervosa (42). The exact mechanism of reduced IFNγ production in obesity has not been clarified, but studies indicate the likelihood of a primary hypo-responsiveness of IFNγ-producing cells when stimulated directly with mitogens (37,38) or IL-18 (36). Our data suggest that iron depletion, as measured by sTfR, may be associated with *ex vivo* ZY stimulated IFNγ production in obesity. In a group of non-obese children, iron deficiency was associated with decreased infection and increased formation of IFNγ compared to children with normal iron status (13). Additionally, iron chelation therapy in children with malaria resulted in higher levels of Th1 cytokines and nitric oxide *in vivo* compared to children not receiving chelation therapy (14–16). Together these findings suggest, iron deficiency may strengthen T-cell-mediated immune function through increased Th1 cytokine formation, including IFNγ, in an inflamed milieu such as that observed in

obesity (11). Interestingly, following substantial weight loss, decreased inflammation, and improved iron status, *ex vivo* basal and stimulated IFNγ from WB cultures was not significantly improved in the obese women. This suggests that the persistent inflammation and obesity in these individuals, coupled with improved iron status, may be sufficient to perpetuate reduced IFNγ expression, although, additional studies are necessary to confirm this hypothesis.

Serum hepcidin was negatively correlated with *ex vivo* LPS and ZY simulated IFNγ production at baseline in the obese women while significant weakening of this relationship was observed following weight reduction. A recent study conducted in mice suggests that hepcidin may play a key role in modulating the acute phase response through upregulation of SOCS3 (43). Pre-treatment of cultured macrophages with hepcidin resulted in increased SOCS3 production and reduced expression of both IL-6 and TNFα following LPS injection; IFNγ response was not assessed. Also, mice injected with hepcidin were able to suppress acute inflammation following a dose of LPS. Despite these findings we did not see a relationship between serum hepcidin and LPS or ZY stimulated IL-6 or TNFα in the obese women. Collectively these findings suggest that further exploration and understanding of the interaction between obesity, iron status, hepcidin, and immunological function, particularly IFNγ, is warranted.

Our study had several limitations. First, the sample size was relatively small and consisted of only morbidly obese premenopausal women, which limited the generalizability of our findings. Future studies with larger more diverse populations are needed. In addition, the mechanisms linking altered *ex vivo* cytokine production with obesity, hepcidin, and iron status were not investigated, the range of microbial stimuli evaluated was limited to LPS and ZY and the technique used (WB cultures) did not allow us to discriminate which cell populations were responsible for production of each cytokine.

In conclusion, *ex vivo* LPS and ZY stimulated cytokine production from WB cultures was altered in premenopausal women with morbid obesity. Significant weight loss resulted in the normalization of some but not all observed alterations. Furthermore, iron status and serum hepcidin were associated with *ex vivo* LPS and ZY stimulated IFNγ in obesity. These findings warrant additional studies aimed at investigating the mechanisms of the association between obesity, iron status, hepcidin and cytokine production as well as the potential clinical implications of these findings in terms of susceptibility to infection and autoimmune diseases as well as response to vaccination.

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Table 1

Clinical and biochemical characteristics and whole blood cytokine stimulation of obese and non-obese women at baseline and at six months post-surgery in the obese women

Variable	Obese Baseline[a] (n=17)	Non-obese Baseline[a] (n=19)	Obese Six-months Post-surgery[a] (n=17)
BMI (kg/m2)[b,d]	46.6 (7.9)	22.5 (3.0)[f]	37.6 (5.5)[g]
Waist circumference (cm)[b,d]	128.2 (17.6)	78.5(6.7) [f]	114.5 (15.6) [g]
Serum IL-6 (pg/mL)[c,d]	2.85 (1.18)	2.44 (2.11)	1.66 (0.95) [g]
hs-CRP (mg/L)[c,d]	11.88 (5.53)	0.91 (1.45) [f]	6.67 (4.77) [g]
HOMA-*IR* [c,e]	4.97 (3.72)	1.44 (0.66) [f]	2.62 (2.08) [g]
Hemoglobin (g/dL) [b,d]	12.1 (1.14)	12.1 (0.9)	13.0 (1.1) [g]
Serum Hepcidin (ng/mL)[c,d]	123.27 (84.76)	22.56 (1.30) [f]	31.64 (23.69) [g]
sTfR (nmol/L)[b,e]	33.17 (11.66)	28.57 (13.88)	24.28 (6.44) [g]
IL-6 (pg/mL) (control)[c,e]	312.85 (473.46)	302.03 (369.06)	864.33 (489.93) [g]
IL-6 LPS (pg/mL) [b,d]	8288.56 (3606.74)	11999.55 (2881.20) [f]	12071.76 (4073) [g]
IL-6 ZY (pg/mL)[b,d]	7320.40 (5054.52)	11000.62 (3712.67) [f]	11846.70 (4888) [g]
IL-10 (pg/mL) (control)[c,e]	477.70 (368.57)	470.64 (313.81)	473.95 (356.49)
IL-10 LPS (pg/mL) [b,d]	4375.09 (2369.51)	5242.83 (1828.11)	4438.09 (2058)
IL-10 ZY (pg/mL) [b,d]	4870.36 (2434.49)	4886.55 (2114.54)	4073.43 (2256)
IL-22 (pg/mL) (control)[c,e]	132.96 (88.61)	96.26 (50.99)	160.79 (175.84)
IL-22 LPS (pg/mL) [b,e]	501.96 (196.75)	343.09 (125.07) [f]	563.62 (520.18)
IL-22 ZY (pg/mL)[c,e]	469.65 (145.97)	361.59 (154.77) [f]	647.28 (770.18)
IFNγ (pg/mL) (control)[c,e]	365.27 (305.51)	985.41 (351.05) [f]	426.68 (410.79)
IFNγ LPS (pg/mL)[c,e]	798.94 (812.58)	2526.84 (637.71) [f]	1053.22 (1190)
IFNγ ZY (pg/mL)[c,e]	771.71 (745.92)	2403.96 (741.70) [f]	976.33 (1146)
TNFα (pg/mL) (control) [b,d]	14.00 (6.83)	12.27 (8.34)	19.74 (10.56)
TNFα LPS (pg/mL) [b,d]	63.59 (25.04)	110.60 (62.50) [f]	161.47 (123.29) [g]
TNFα ZY (pg/mL) [b,e]	51.05 (23.56)	92.32 (55.81) [f]	109.21 (76.79) [g]

[a] Values are mean (± standard deviation)

[b] T-test was used for comparison of obese versus non-obese women at baseline

[c] Wilcoxon rank sum (non-parametric test) was used for comparison of obese versus non-obese women at baseline

[d] Paired t-test was used for comparison of baseline and follow-up values in the obese women

[e] Wilcoxon signed rank (non-parametric test) was used for comparison of baseline and follow-up values in the obese women

[f] denotes p<0.025 (correction factor= 0.05/2 groups) for comparison between obese vs. non-obese women at baseline

[g] denotes p<0.025 (correction factor= 0.05/2 groups) for comparison between obese at baseline vs. six months post-restrictive bariatric surgery

Abbreviations: BMI, body mass index; IL-6, interleukin-6; hs-CRP, high sensitivity C reactive protein; HOMA-*IR*, homeostasis model assessment of insulin resistance; sTfR, serum transferring receptor; control, unstimulated whole blood culture, ; LPS, lipopolysacchride; ZY, zymosan; IL-10, interleukin-10; IL-22, interleukin-22; IFNγ, interferon gamma; TNFα, tumor necrosis alpha

Table 2

Bivariate correlations between whole blood stimulated IFNγ[a] and anthropometric and biochemical variables in obese and non-obese premenopausal women at baseline

	Obese (n=17		Non-obese (n=19)	
	IFNγ LPS	**IFNγ ZY**	**IFNγ LPS**	**IFNγ ZY**
BMI (kg/m2)	−0.16	−0.06	−0.09	−0.28
Waist Circ. (cm)	−0.17	−0.09	−0.07	−0.38
Serum IL-6 (pg/mL)	−0.14	0.18	−0.23	−0.31
CRP (mg/L)	−0.13	0.13	0.11	0.21
HOMA-*IR*	−0.03	0.18	0.08	0.14
Hemoglobin (g/dL)	−0.31	0.20	0.01	0.33
Serum hepcidin (ng/mL)	−0.61[b]	−0.63[b]	0.21	0.30
sTfR (nmol/L)	0.47	0.58[b]	−0.08	−0.17

[a] units are in pg/mL

[b] p <0.025 (correction factor= 0.05/2 groups)

Abbreviations: BMI, body mass index; IL-6, interleukin-6; CRP, high sensitivity C reactive protein; HOMA-*IR*, homeostasis model assessment of insulin resistance; sTfR, serum transferring receptor; IFNγ, interferon gamma LPS, lipopolysacchride; ZY, zymosan

Table 3

Bivariate correlations between whole blood stimulated IFNγ[a] and anthropometric and biochemical variables in obese women six months post-restrictive bariatric surgery (n=17)

	IFNγ LPS 6m	IFNγ ZY 6m	IL6 LPS 6m	IL6 ZY 6m	TNFα LPS 6m	TNFα ZY 6m
BMI (kg/m2), 6m	−0.23	−0.36	−0.17	0.05	−0.29	−0.13
Waist Circ.(cm), 6m	−0.50[b]	−0.53[b]	−0.21	0.17	−0.28	0.06
Serum IL-6 (pg/mL), 6m	0.11	0.03	−0.30	−0.41	−0.62 [b]	−0.36
CRP (mg/L), 6m	0.10	−0.07	0.02	0.02	−0.35	−0.23
HOMA-*IR*	−0.15	−0.13	0.10	0.05	0.07	0.04
Hemoglobin (g/dL), 6m	−0.54[b]	−0.51	−0.10	0.42	0.06	0.37
Serum hepcidin (ng/mL), 6m	−0.21	−0.15	−0.17	−0.05	−0.13	−0.18
sTfR (nmol/L), 6m	0.28	0.15	0.05	−0.04	−0.12	−0.20

[a] units are in pg/mL

[b] p <0.05

Abbreviations: BMI, body mass index; IL-6, interleukin-6; hs-CRP, high sensitivity C reactive protein; HOMA-*IR*, homeostasis model assessment of insulin resistance; sTfR, serum transferring receptor; IFNγ, interferon gamma LPS, lipopolysacchride; ZY, zymosan; TNFα, tumor necrosis alpha