International Journal of Basic and Applied Medical Sciences ISSN: 2277-2103 (Online)
An Open Access, Online International Journal Available at http://www.cibtech.org/jms.htm
2015 Vol. 5 (2) May-August, pp. 92-97/Ghosh et al.
Research Article

ASSOCIATION OF INTERFERON-GAMMA AN INFLAMMATORY MARKER AND FASTING SERUM BILIRUBIN AN OXIDATIVE STRESS MARKER IN ESTABLISHED CASES OF METABOLIC SYNDROME AROUND KOLKATA AND ITS NEIGHBORHOODS

***Sandip Ghosh[1], Debjyoti Bhattacharya[2], Chittaranjan Maity[1] and Gora Chand Bhattacharya[3]**
[1]*Department of Biochemistry, KPC Medical College, Jadavpur, Kolkata, India*
[2]*Department of Biochemistry, Calcutta National Medical College, Kolkata, India*
[3]*Department of Biochemistry, MGM Medical College & LSK Hospital, Kishanganj 855107, Bihar, India*
**Author for Correspondence*

ABSTRACT
Subjects of Metabolic Syndrome are prone to develop risk factors for type 2 diabetes mellitus and cardiovascular diseases. . Inflammatory process and atherosclerosis are associated with the disorder. In this study, serum interferon gamma a potent inflammatory marker and serum total bilirubin an antioxidant, have been measured in 150 subjects of Metabolic Syndrome and compared with 150 controls. We observed an inverse relationship between interferon gamma and total bilirubin. The subjects of metabolic syndrome were diagnosed as per NCEP (ATP III) criteria BMI, waist –hip ratio, lipid profile and HOMA- IR of the test and control groups have been considered for diagnostic criteria.

Keywords: *Metabolic Syndrome, Gamma Interferon, Serum Bilirubin*

INTRODUCTION
Metabolic syndrome or Syndrome –X is recognized as one of the most serious risk factors for developing type II diabetes, as well as cardiovascular disease (Wilson *et al.,* 2005). Criteria for diagnosis of metabolic syndrome are the criteria of the National Cholesterol Education Programme (NCEP) Adult Treatment Panel III (ATP-III) (Grundy, 2008; Eckel *et al.,* 2005; NCEP, 2001), with combination of body mass index (BMI) and waist circumference. The key points of this criteria are: 1) triglyceride levels ≥ 150 mg/dl ;2) HDL – C levels < 40 mg/dl in men or < 50 mg/dl in women; 3) Fasting plasma glucose levels ≥ 110 mg/dl or taking an anti diabetic medication; 4) systolic blood pressure ≥ 130 mmHg or diastolic blood pressure ≥ 85 mmHg (or receiving drug therapy for hypertension); and 5) BMI > 25 Kg/Meter ² (Ishizaka *et al.,* 2005).

The exact mechanism underlying metabolic syndrome has not yet been elucidated completely. Many cross- sectional or longitudinal studies have shown that metabolic syndrome is strongly associated with inflammation (Wang *et al.,* 2004; Rutter *et al.,* 2004; Festa *et al.,* 2000), insulin sensitivity (Lann and LeRoith, 2007), endothelial dysfunction (Gideon *et al.,* 2007). As a result, several biological markers have been proposed as risk factors for metabolic syndrome that are associated with an increase in the risk of metabolic syndrome: homeostasis model assessment insulin resistance index (Festa *et al.,* 2000); (HOMA-IR; as a marker of insulin resistance), homocysteine (Gideon *et al.,* 2007) (as a marker of endothelial dysfunction). Gamma interferon was described by E. F. Wheelock as a product of human leukocytes when stimulated with phytohemagglutinin an extract of the kidney beans *Phaseolus vulgaris* (Wheelock, 1965), and by others as a product of antigen-stimulated lymphocytes (Green *et al.,* 1969) or tuberculin-sensitized mouse peritoneal lymphocytes (Milstone and Waksman, 1970); the resulting supernatants were shown to inhibit growth of vesicular stomatitis virus. Those reports also contained the basic observation underlying the now widely employed interferon gamma release assay used to test for tuberculosis. In humans, the IFNγ protein is encoded by the *IFNG*gene (Naylor *et al.,* 1983). IFNγ was formerly known as immune IFN, macrophage activating factor, Tcell IFN, and type II IFN. IFNγ is synthesized by activated T cells (CD4Th0, CD4Th1 and CD8) and NK cells. IFNγ is produced by CD4 Th1 cells following antigen activation (Mytar *et al.,* 1995). Interferon gamma (IFNγ) is a dimerized soluble cytokine that is the only member of the type II class of interferons (Gray and

Goeddel, 1982). IFNγ is also produced as an aberrant expression of class II histocompatibility antigens by tissue cells (such as thyroid cells) that do not normally express them leading to autoimmune disease (Dorland, 2003). It is an important cytokine produced by body in altered adipose tissue metabolism (Viviane *et al.,* 2008). IFNγ and growth factors (such as FGF) belong to distinct groups regarding their regulation by heparan sulfate/heparin-like molecules (Sadir *et al.,* 1998). C terminal residues of IFNγ binds to heparan sulfate by electrostatic interactions between lysine or arginine charged side chains and heparan sulfate sulphate groups and it also requires an octasaccharide for effective binding (Vanhaverbeke *et al.,* 2004). As there is no available data for study in Indian population, the object of present study is to establish any co-relation between interferon-gamma an inflammatory marker and fasting serum bilirubin an oxidative stress marker in established cases of metabolic syndrome around Kolkata and neighborhoods in India.

As bilirubin acts as a potent antioxidant (Stocker *et al.,* 1987), the object of study is to establish any correlation between gamma IFN interferon and fasting serum bilirubin in proved cases of metabolic syndrome among the patients visiting outpatient departments of Calcutta National Medical College, Kolkata, India. Informed consent was duly taken from each subject under study, and the entire procedure was done as per the Institutional ethical permission.

Inclusion Criteria: In this hospital based cross sectional study, subjects of metabolic syndrome were diagnosed with high BMI, waist-hip ratio, HOMA-IR, hypertension, known type-2 diabetes mellitus. Healthy controls were chosen from the spouse of metabolic syndrome patients, medical students, teaching and non-teaching staffs of the Calcutta National Medical College, Kolkata.

Exclusion criteria: Subjects with chronic diseases, known tuberculosis, hepatitis due to any cause, Cushing's syndrome, pregnancy, chronic alcoholism, renal dysfunction, long treated diabetes mellitus, taking treatment for thyroid disorders, recent history of fever and infection were excluded from the study.

MATERIALS AND METHODS

Blood samples were collected from the patients visiting the outpatient department of Calcutta National Medical College, Kolkata, India. Necessary clearance from Institutional ethical Committee was obtained. Blood was collected from ante cubital vein. Cells were separated by centrifugation at 5000 rpm for 5 minutes. Blood glucose, serum bilirubin were estimated immediately. Serum was separated and stored in -20^0C for future use. Patients Body Mass Index was measured using standard measuring tape and weighing machine. Height was measured against wooden vertical scale. Body mass index was measured using the following formula mass in Kg/ height in meter^2. Fasting plasma glucose and total bilirubin was estimated using autoanalyser (A125-Transasia). HbA1c was estimated using Bio-Rad D-10. Fasting insulin was estimated using ELISA kit of Monobind Inc. waist –hip ratio was measured using standard measuring tape in the outpatient department. Insulin resistance was estimated by calculating HOMA-IR originally described by Mathew *et al.,* (1985) (Homeostatic model assessment- Insulin resistance). HOMA-IR was calculated using the following formula:

HOMA-IR= fasting glucose (mg%) × fasting insulin (μU/ml)/405.

Gamma interferon was estimated using ELISA kit of Ray Biotech in TECAN ELISA reader, Imperial Biotech. Subjects were divided in two groups - control and test. Control group had 150 healthy individuals and test group had 150 proved cases of metabolic syndrome. Test group subjects were selected using standard criteria for metabolic syndrome. Individuals with chronic disease manifestation were excluded from study. All reagents and samples were brought to room temperature (18 - 25ºC) before use. A standard graph was prepared with standards provided with the kit. Standards were diluted by serial dilution method. In case of controls eight obese and eight non obese subjects were tested with duplicates. 100 µl of each standard and sample into appropriate wells. Wells were covered and incubated for 2.5 hours at room temperature with gentle shaking. The solution was then discarded and washed four times by automated ELISA plate washer with 1X wash solution. After washing traces of wash solution was removed by gentle tapping against blotting paper towel. 100 µl of 1X prepared biotinylated antibody was added to each well and incubated for 1 hour at room temperature with gentle shaking in a motorized

shaker. Solution was discarded. Wash procedure was repeated as before. 100 µl of prepared Streptavidin solution was added to each well and incubated for 45 minutes at room temperature with gentle shaking. The solution was discarded and the wash procedure was repeated as before. 100 µl of TMB One-Step Substrate Reagent was added to each well and incubated for 30 minutes at room temperature in the dark with gentle shaking. 50 µl of Stop Solution was added to each well. ELISA plate was read at 450 nm immediately using plate reader.

Data was analyzed in Microsoft Excel and SPSS software. Value of individual parameter was expressed as mean ± standard deviation. Significance of difference of the means within the groups was tested by unpaired Student's t- test. Every where $P<0.05$ was considered as significant.

RESULTS AND DISCUSSION

Results

Analysed data are furnished in the tables below. Table-1 shows the physical parameters including HOMA-IR of control and test group subjects. All the subjects were euthyroids and were not reported to have history of any chronic disease. Obesity markers were also estimated which are not included in this article. Table -1 shows clear evidence that there is no significant difference in the average age of the subjects. Male and female ratios of the subjects are also close among the groups.

Table 1: Age and gender distribution in sample population with physical parameters

Parameter	Control Group N=150	Test Group n=150
Age in years	43.42±12.84	44.71±11.2
Male	88	82
Female	62	68
Waist/Hip ratio	0.82±0.03	0.95±0.05
HOMA-IR Score	1.148±0.35	2.34±2.0

Table 2: Special parameters of metabolic syndrome and control subjects (n=150)

Parameter	Unit	Control		Test		P value
		Mean	±sd	Mean	±sd	
Fasting Plasma Glucose	mg/deciliter	85.59	2.9	178.6	13.84	<0.0001
Fasting Insulin	mIU/milliliter	5.43	1.67	5.37	4.65	0.8683
Body Mass Index	Kg/meter^2	23.29	3.9	36.31	4.5	<0.0001
HbA1c	NGSP (%)	5.7	0.14	10.43	0.87	<0.0001
Serum Gamma Interferon	picogram/milliliter	6.38	2.03	11.19	0.86	<0.0001
Serum Total Bilirubin	mg/deciliter	0.74	0.11	0.38	0.07	<0.0001

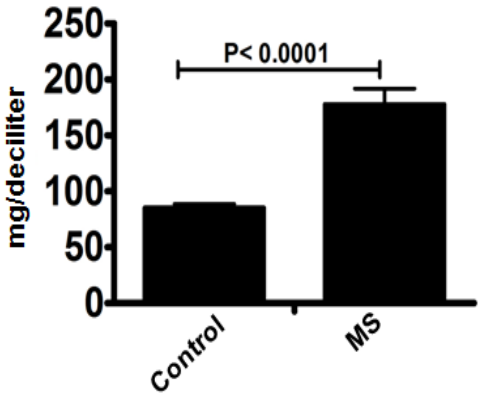

Figure 1: Fasting Plasma Glucose levels

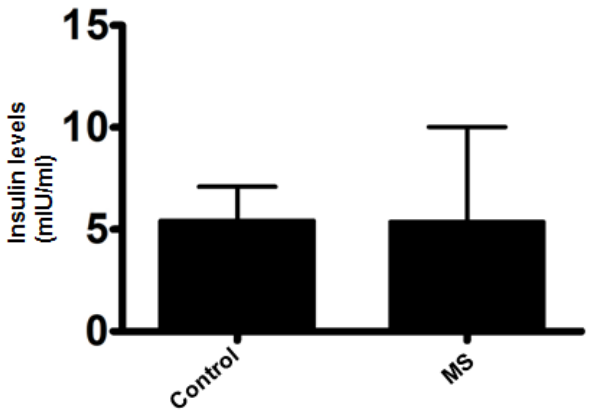

Figure 2: Fasting Insulin Levels

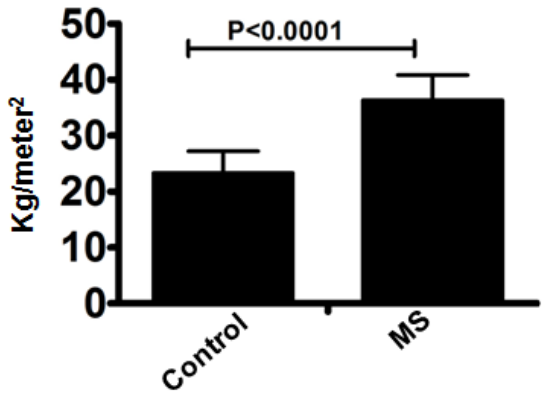

Figure 3: Body Mass Index

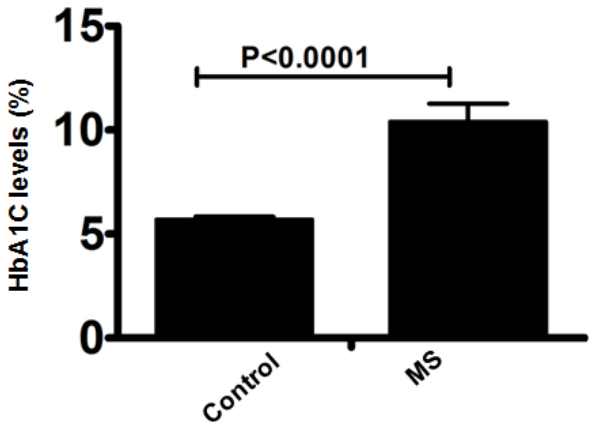

Figure 4: Plasma HbA1C levels

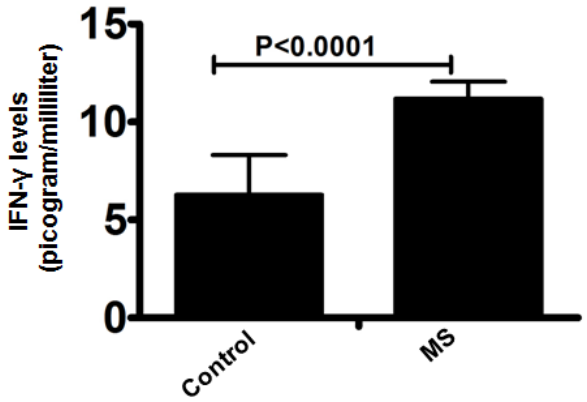

Figure 5: IFN-γ levels

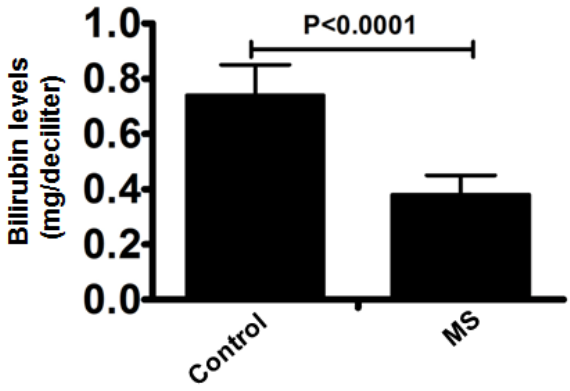

Figure 6: Fasting Total Bilirubin levels

Waist –hip Ratio: waist –hip ratio is an important parameter for assessment of obesity (**WHO Report, 2012**). WHR was found to be 0.82±0.03 and 0.95±0.05 in control and metabolic syndrome groups respectively (Table 1).

HOMA IR Score: control and metabolic syndrome groups were found to have significantly different HOMA IR score i.e., 1.148±0.35 and 2.34±2.0 respectively. The *p* value is <0.0001 (Table 1).

Fasting Plasma Glucose: Mean and SD of fasting plasma glucose of control group was 85.59±2.9 mg/deciliter. Mean and SD of fasting plasma glucose of metabolic syndrome group was 178.6±13.84 mg/deciliter. The *p* value is <0.0001 (Table 2, figure 1).

Fasting Insulin: Mean and SD of fasting insulin of control group was 5.43±1.67 mIU/mililitre. Mean and SD of fasting insulin of metabolic syndrome group was 5.37±4.65 mIU/milliliter. The *p* value is <0.8683 (Table 2, figure 2). HOMA-IR is better parameter than fasting insulin for detection of metabolic syndrome cases (Matsuba *et al.*, 2012).

Body Mass Index: Mean and SD of body mass index of control group was 23.29±3.9 Kg/Meter^2. Mean and SD of body mass index of metabolic syndrome group was 36.31±4.5 Kg/Meter^2. The *p* value is <0.0001 (Table 2, figure 3).

HbA1c: Mean and SD of HbA1c of control group was 5.7±0.14 %. Mean and SD of HbA1c of metabolic syndrome group was 10.43±0.87 %. The *p* value is <0.0001. HbA1c or A1c is a diagnostic as well as prognostic parameter for of duration of hyperglycemic state of an individual (American Diabetes Association, 2011) (Table 2, figure 4).

Serum Gamma Interferon: Mean and SD of Serum Gamma Interferon of control group was 6.38±2.03 picogram/milliliter. Mean and SD of Serum Gamma Interferon of metabolic syndrome group was 11.19±0.86 picogram/milliliter. The *p* value is <0.0001 (Table 2, figure 5).

Serum Total Bilirubin: Mean and SD of Serum Total Bilirubin of control group was 0.74±0.11 mg/deciliter. Mean and SD of Serum Total Bilirubin of metabolic syndrome group was 0.38±0.07 mg/deciliter. The *p* value is <0.0001 (Table 2, figure 6).

Discussion

According to NCEP (ATP 111) protocol individuals of study group are diagnosed as metabolic syndrome subjects. There is clear evidence that two groups of subjects are significantly distinct. Fasting plasma glucose, HOMA-IR, waist-hip ratio, BMI, HbA1c, interferon gamma and fasting serum total bilirubin levels are significantly distinct between control and study group each parameter is having p value <0.05. There is a report of inverse relationship of fasting serum bilirubin and metabolic syndrome in Korean population (Hwang and Kim, 2010; Choi *et al.*, 2013). Our study also includes a new finding that gamma interferon, a potent inflammation marker has an inverse relationship with fasting serum total bilirubin. Metabolic syndrome is a condition in which there is increase of oxidative stress in the affected subjects. Researchers from other part of the globe also reported increased oxidative stress in metabolic syndrome (Ishizaka *et al.*, 2005; Rutter *et al.*, 2004; Festa *et al.*, 2000; Gideon *et al.*, 2007). Bilirubin functions as an anti-oxidant in association with glutathione (Sedlak *et al.*, 2009). We propose that decrease in fasting serum total bilirubin may be caused by increased oxidative stress in metabolic syndrome.

REFERENCES

American Diabetes Association (2011). Standards of medical care in diabetes-2011. *Diabetes Care* **34** S11-S66.

Choi SH, Yun KE and Choi HJ (2013). Relationships between serum total bilirubin levels and metabolic syndrome in Korean adults. *Nutrition, Metabolism and Cardiovascular Diseases* **23**(1) 31-37.

Dorland WA (2003). *Dorland's Illustrated Medical Dictionary*, 30th edition (Philadelphia: WB Saunders).

Eckel RH, Grundy SM and Zimmet PZ (2005). The metabolic syndrome. *Lancet* **365** 1415 – 1428.

Festa A, D'Agostino R Jr, Howard G, Mykkänen L, Tracy RP and Haffner SM (2000). Chronic subclinical inflammation as part of the insulin resistance syndrome: the Insulin Resistance Atherosclerosis Study (IRAS). *Circulation* **102**(1) 42-47.

Gideon R Hajer, Yolanda van der Graaf, Jobien K Olijhoek, Marianne C Verhaar and Frank LJ Visseren (2007). For the SMART Study Group. Levels of homocysteine are increased in metabolic syndrome patients but are not associated with an increased cardiovascular risk, in contrast to patients without the metabolic syndrome. *Heart* **93**(2) 216–220.

Gray PW and Goeddel DV (1982). Structure of the human immune interferon gene. *Nature* **298**(5877) 859–63, doi:10.1038/298859a0, PMID 6180322.

Green JA, Cooperband SR and Kibrick S (1969). Immune specific induction of interferon production in cultures of human blood lymphocytes. *Science* **164**(3886) 1415–1417.

Grundy SM (2008). Metabolic syndrome pandemic. *Arteriosclerosis Thrombosis Vascular Biology* **28** 629-636.

Hwang HJ and Kim SH (2010). Inverse relationship between fasting direct bilirubin and metabolic syndrome in Korean adults. *Clinica Chimica Acta* **411** 1496–1501.

Ishizaka N, Ishizaka Y, Toda E, Nagai R and Yamakado M (2005). Association between serum uric acid, Metabolic Syndrome and carotid Atherosclerosis in Japanese individuals. *Arteriosclerosis Thrombosis Vascular Biology* **25** 1038 – 1044.

Lann D and Le Roith D (2007). Insulin resistance as the underlying cause for the metabolic syndrome. *Medical Clinics of North America* **91** 1063 – 1077.

Matsuba I *et al.*, (2012). Insulin Levels and Metabolic Risk Factors in Type 2 Diabetic Patients at the First Visit in Japan. *Diabetes Care* **35** 1853–1857.

Matthews DR, Hosker JP, Rudenski AS, Naylor BA, Treacher DF and Turner RC (1985). Homeostasis model assessment: Insulin resistance and beta-cell function from fasting plasma glucose and insulin concentrations in man. *Diabetologia* **28** 412–419.

Milstone LM and Waksman BH (1970). Release of virus inhibitor from tuberculin-sensitized peritoneal cells stimulated by antigen. *Journal of Immunology* **105**(5) 1068–1071.
Mytar B, Woloszyn M and Ruggiero I *et al.*, (1995). Monocyte mediated regulation of antigen driven by IFN gamma by T cells, the role of endogenously produced TNF. *Immunological investigations* **24** 897-906.
Naylor SL, Sakaguchi AY, Shows TB, Law ML, Goeddel DV and Gray PW (1983). Human immune interferon gene is located on chromosome 12. *Journal of Experimental Medicine* **157**(3) 1020–7.
NCEP (2001). Executive summary of the third report of the National Cholesterol Education Programme (NCEP), Expert panel on detection, evolution, and treatment of high blood cholesterol in adults (Adult treatment panel III). *Journal of American Medical Association* **285** 2486-2497.
Rutter MK, Meigs JB, Sullivan LM, D'Agostino Sr RB and Wilson PW (2004). C-reactive protein, the metabolic syndrome, and prediction of cardiovascular events in the Framingham Offspring Study. *Circulation* **110**(4) 380-385.
Sadir R, Forest E and Lortat-Jacob H (1998). The heparan sulfate binding sequence of interferon-gamma increased the on rate of the interferon-gamma-interferon-gamma receptor complex formation. *Journal of Biological Chemistry* **273**(18) 10919–10925.
Sedlak TW, Saleh M, Higginson DS, Paul BD, Juluri KR and Synder SH (2009). Bilirubin and glutathione have complementary antioxidant and cytoprotective roles. *Proceedings of the National Academy of Sciences USA* **106** 5171-5176.
Stocker R, Yamamoto Y, McDonagh AF, Glazer AN and Ames BN (1987). Bilirubin is an antioxidant of possible physiological importance. *Science* **235**(4792) 1043-1046.
Vanhaverbeke C, Simorre JP, Sadir R, Gans P and Lortat-Jacob H(2004). NMR characterization of the interaction between the C-terminal domain of interferon-γ and heparin-derived oligosaccharides. *Biochemical Journal* **384**(Pt 1) 93–99.
Viviane Zorzanelli Rocha *et al.*, (2008). Interferon-gamma, a Th1 Cytokine, Regulates Fat Inflammation A Role for Adaptive Immunity in Obesity. *Circulation Research* **103** 467-476; originally published online July 24, 2008; doi: 10.1161/CIRCRESAHA.108.177105.
Wang YY, Lin SY, Liu PH, Cheung BM and Lai WA (2004). Association between hematological parameters and metabolic syndrome components in a Chinese population. *Journal of Diabetes Complications* **18** 322 – 327.
Wheelock EF (1965). Interferon-like virus-inhibitor induced in human leukocytes by phytohemagglutinin. *Science* **149**(3681) 310-311.
WHO Report (2012). Waist Circumference and Waist-Hip Ratio, Report of a WHO Expert Consultation. World Health Organization, 8–11 December 2008, Retrieved March 21, 2012.
Wilson PW, D'Agostino RB, Parise H, Sullivan L and Meigs JB (2005). Metabolic syndrome as a precursor of cardiovascular disease and type-II diabetes mellitus. *Circulation* **112** 3066-3072.