



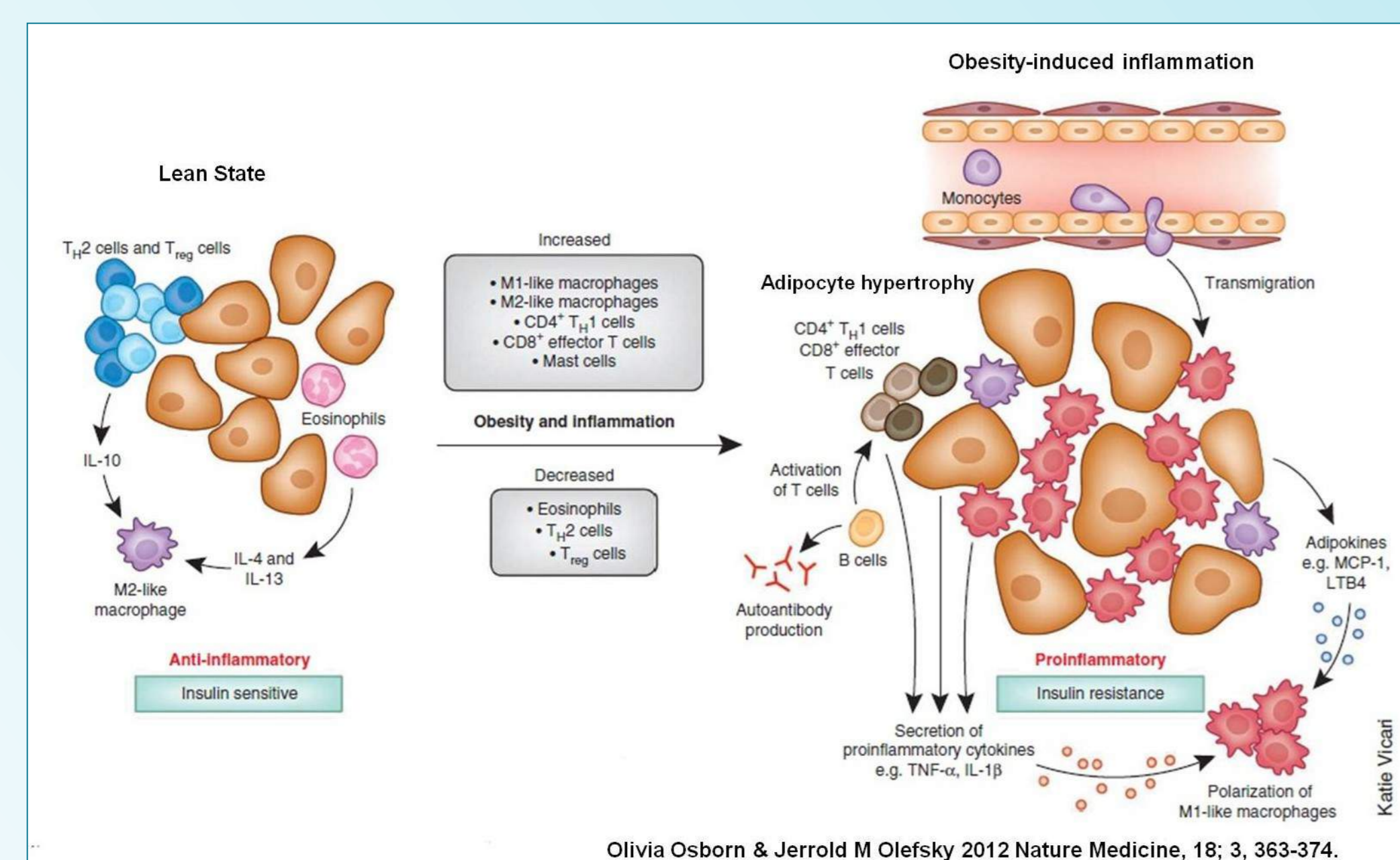
# Pro-inflammatory (M1) and anti-inflammatory (M2) profiles in adipose tissue of lean and obese children and adolescents.

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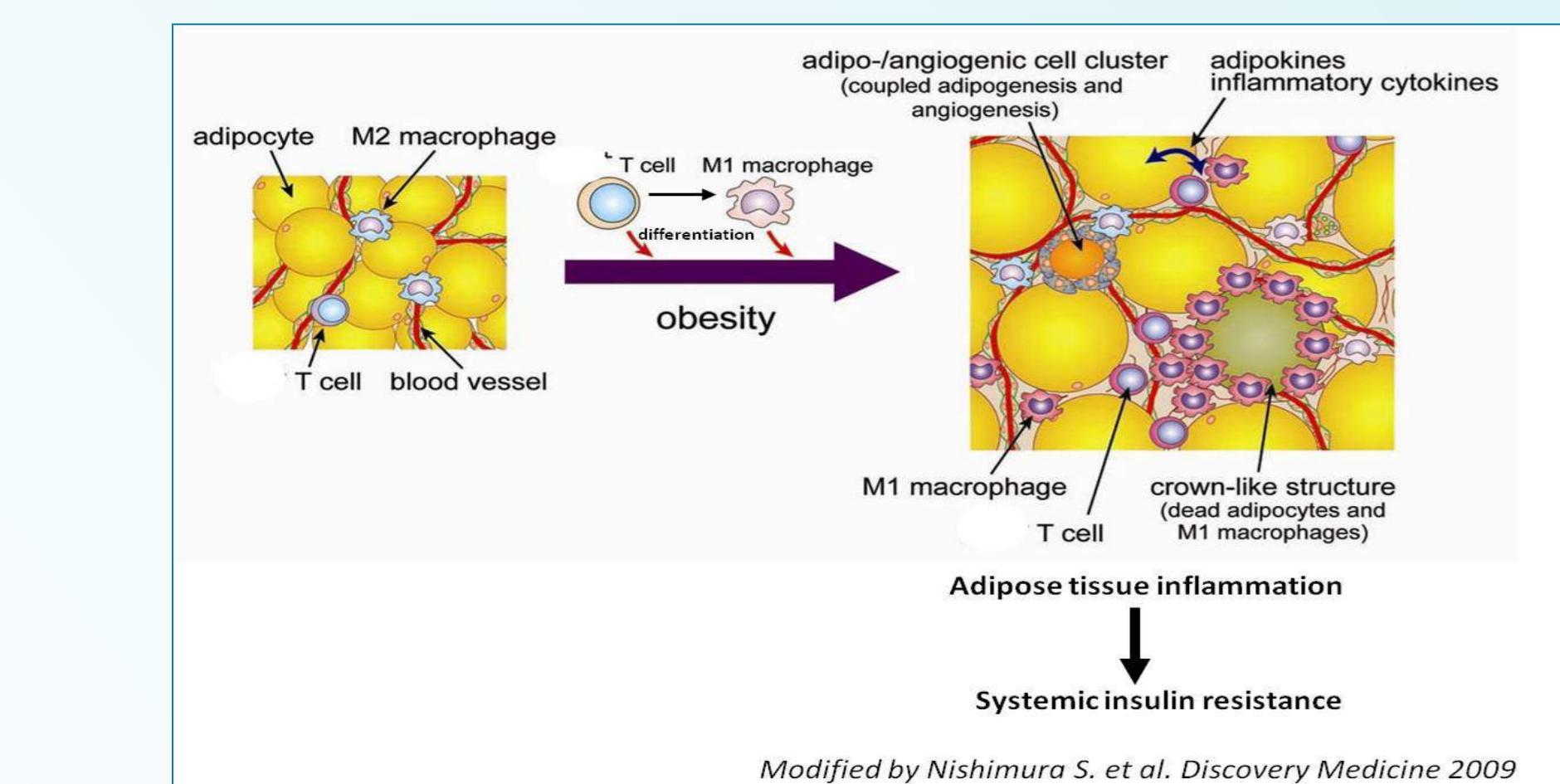
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## Introduction

- Childhood obesity predisposes to metabolic disorders, with white adipocyte inflammation a key underlying pathology.
- Excess energy causes white adipocyte hypertrophy & initial adipose tissue (AT) macrophage infiltration → low grade tissue inflammation.<sup>1</sup>



- Chronic inflammation leads to an unbalanced relationship between M2 (anti-inflammatory) and M1 (pro-inflammatory) macrophages → leading to metabolic complications such as systemic insulin resistance.<sup>1,2</sup>



- The two macrophage activation states M1 (pro-inflammatory) and M2 (anti-inflammatory) exhibit usually "unique" surface markers like CD40 (M1), CD206 (M2) and CD163 (M2).

## Methods

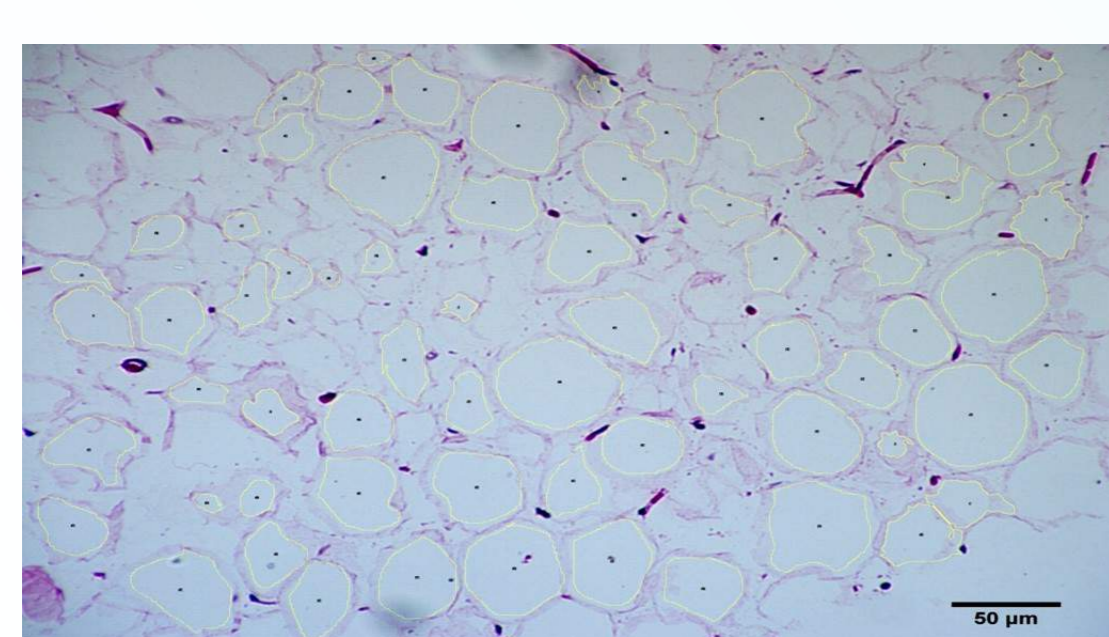
- Paraffin embedded subcutaneous AT microarrays were developed from surgical biopsies of 33 lean (BMI< 85%) & 29 obese (BMI≥95%) pre-pubertal children and adolescents.

- The children were divided into three age- groups:

- Group A:** 2 mos -7 yrs (pre-pubertal)
- Group B:** 8-12 yrs (pre-pubertal)
- Group C:** 10-15 yrs (pubertal)

- The intensity and distribution of CD40, CD163 and CD206 were studied with immunohistochemistry. (positive staining=brown)

- Mean adipocyte size and total adipocyte number were estimated by image analysis (adiposoft).



## Clinical & Biochemical Characteristics

Table 1	Group A		Group B		Group C	
	Lean	Obese	Lean	Obese	Lean	Obese
N	8	8	13	5	13	17
F/M <sup>2</sup>	1/7	1/7	6/7	2/3	2/11	3/14
Tanner	I	I	I	I	II-IV	II-IV
Age (yrs)	2,76 ± 1,94	2,94 ± 1,93	9,55 ± 2,04	10,13 ± 1,62	12,42 ± 0,98	11,45 ± 1,37
BMI (kg/m <sup>2</sup> )	15,99 ± 1,58	18,95 ± 3,71	17,5 ± 2,34	23,38 ± 1,29	20,30 ± 1,90	26,73 ± 4,57
BMISDS <sup>2</sup>	-1 ± 1,31	1,5 ± 0,92*	-0,19 ± 0,99	1,7 ± 0,44*	0,59 ± 0,6	2,25 ± 0,61*
BMI%	26,0 ± 31,45	93,5 ± 3,74**	46,46 ± 27,80	93,2 ± 5,49**	71,0 ± 20,54	95,7 ± 4,21**
WC (cm)	46,0 ± 7,87	53, 83 ± 11,09	60,77 ± 10,28	68,2 ± 13,89	70,46 ± 7,9	77,93 ± 12,8 #
WC%	40,0 ± 29,72	90,0 ± 7,74	68,0 ± 36,9	69,6 ± 40,19	75,77 ± 28,41	90,22 ± 20,12
Birth Weight	3,33 ± 0,93	3,62 ± 0,40	3,24 ± 0,46	2,82 ± 0,58	3,38 ± 0,47	3,55 ± 0,42
Gestational Age						
AGA <sup>4</sup>	4	6	12	3	10	14
SGA <sup>5</sup>	2	0	0	2	0	0
LGA <sup>5</sup>	2	2	1	0	3	3
Family History						
Type 2 Diabetes	1	1	4	1	2	6
Obesity	1	2	4	3	8	7

Data are means ± SD or percentages unless otherwise indicated. \* p < 0.002, \*\* p < 0.001, # p = 0.04 vs respective lean, Abbreviations: <sup>1</sup>Female, <sup>2</sup>Male, <sup>3</sup>SD scores normalized for age and gender, <sup>4</sup>appropriate for gestational age, <sup>5</sup>small for gestational age, <sup>6</sup>large for gestational age

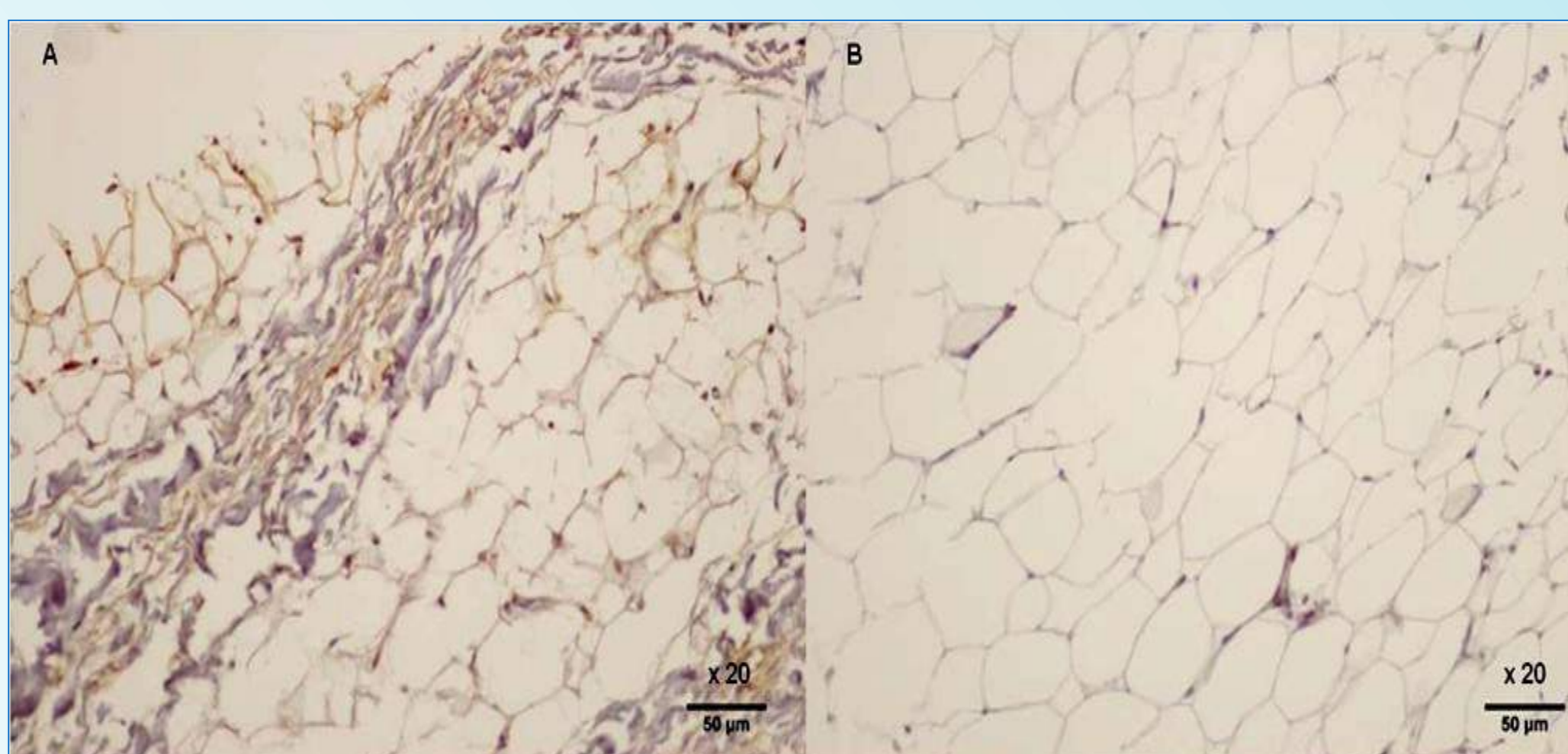
Table 2	Group A		Group B		Group C	
	Lean	Obese	Lean	Obese	Lean	Obese
Glucose (mg/dl)	88,4 ± 5,68	90,5 ± 9,64	96,42 ± 15,5	95,4 ± 7,2	94,1 ± 8,1	97,5 ± 13,20
Total Cholesterol (mg/dl)	155,5 ± 20,41	131,57 ± 20,71	144,25 ± 27,7	145,0 ± 28,4	156,5 ± 27,79	140,0 ± 27,97
HDL (mg/dl)	49,13 ± 13,5	45,3 ± 15,45	55,99 ± 22,25	61,6 ± 13,57	64,92 ± 17,16	53,37 ± 16,32
LDL (mg/dl)	86,75 ± 20,97	74,55 ± 14,29	68,51 ± 12,75	85,7 ± 9,16	68,96 ± 15,04	67,53 ± 14,66
Triglycerides (mg/dl)	67,2 ± 23,7	58,57 ± 28,48	67,54 ± 23,31	59,6 ± 11,5	47,6 ± 15,87	61,5 ± 15,34
Insulin (µU/ml)	2,55 ± 0,59	0,62 ± 0,1*	11,59 ± 6,99	17,19 ± 4,08**	10,76 ± 10,13	15,93 ± 12,76**
HOMA-IR	0,56 ± 0,01	0,13 ± 0,02	2,76 ± 0,27#	4,37 ± 1,36#	2,83 ± 3,00	3,11 ± 2,03#
HMW adiponectin (µg/ml)	8,665 ± 3,95	2,38 ± 0,94*	1,38 ± 0,77§	1,82 ± 0,82	2,21 ± 1,17§	1,51 ± 1,38

Data are means ± SD or percentages unless otherwise indicated. \*p < 0.04 vs respective lean, \*\*p < 0.02 vs obese children of group A, #p < 0.03 vs lean and obese children of group A

Adipocyte Size (µm)	Group A		Group B		Group C	
	Lean	Obese	Lean	Obese	Lean	Obese
Adipocyte Size (µm)	30.31 ± 3.89	32.09 ± 5.07	34.36 ± 9.90	36.44 ± 8.19	39.59 ± 7.56	39.46 ± 0.42
Number of adipocytes per field	131.25 ± 34.23	118.57 ± 49.10	106.1 ± 34.67	96.0 ± 42.01	105.12 ± 21.29	80.25 ± 30.29

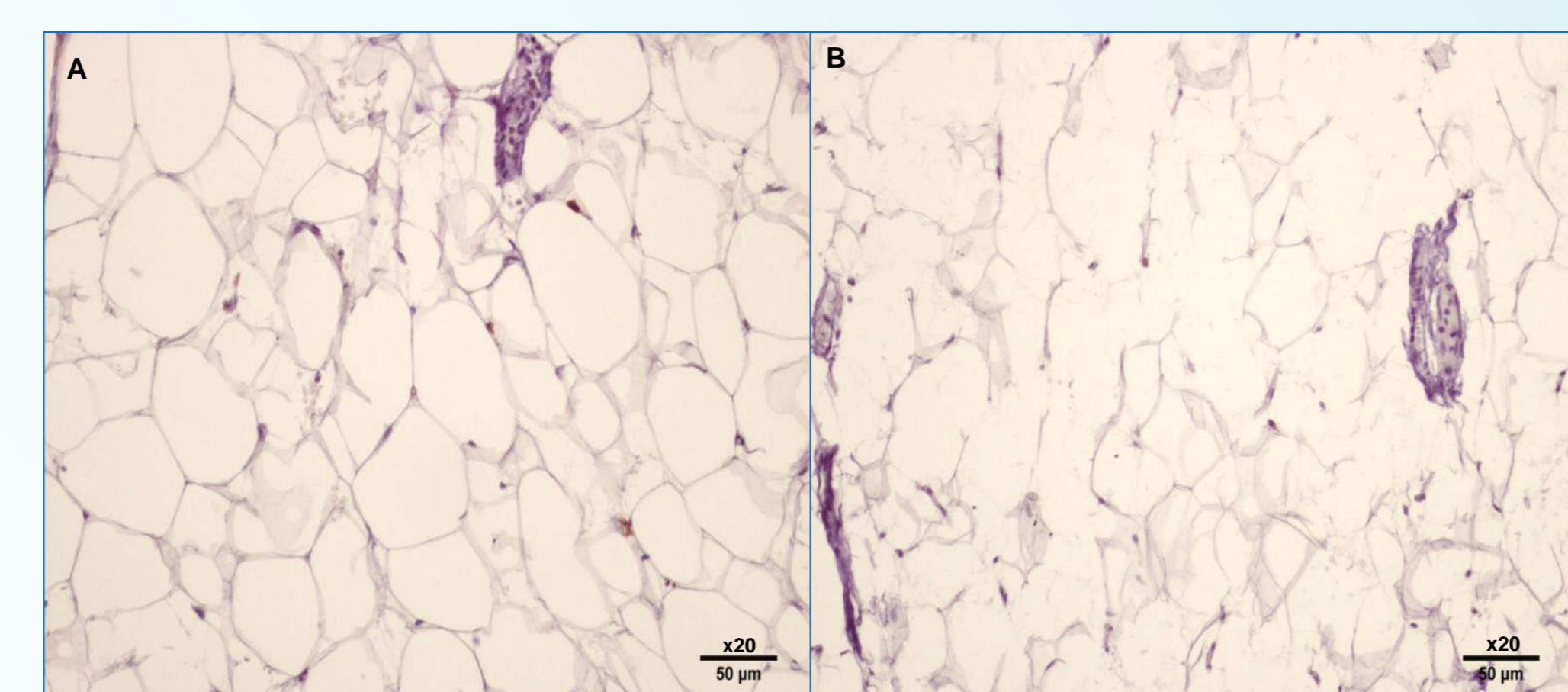
## Results

- CD206 (M2):** Most children of all age groups showed CD206<sup>+</sup> macrophages: Group A lean (A) showed a higher distribution vs their respective obese (B) (p=0.024). (brown = positive staining)

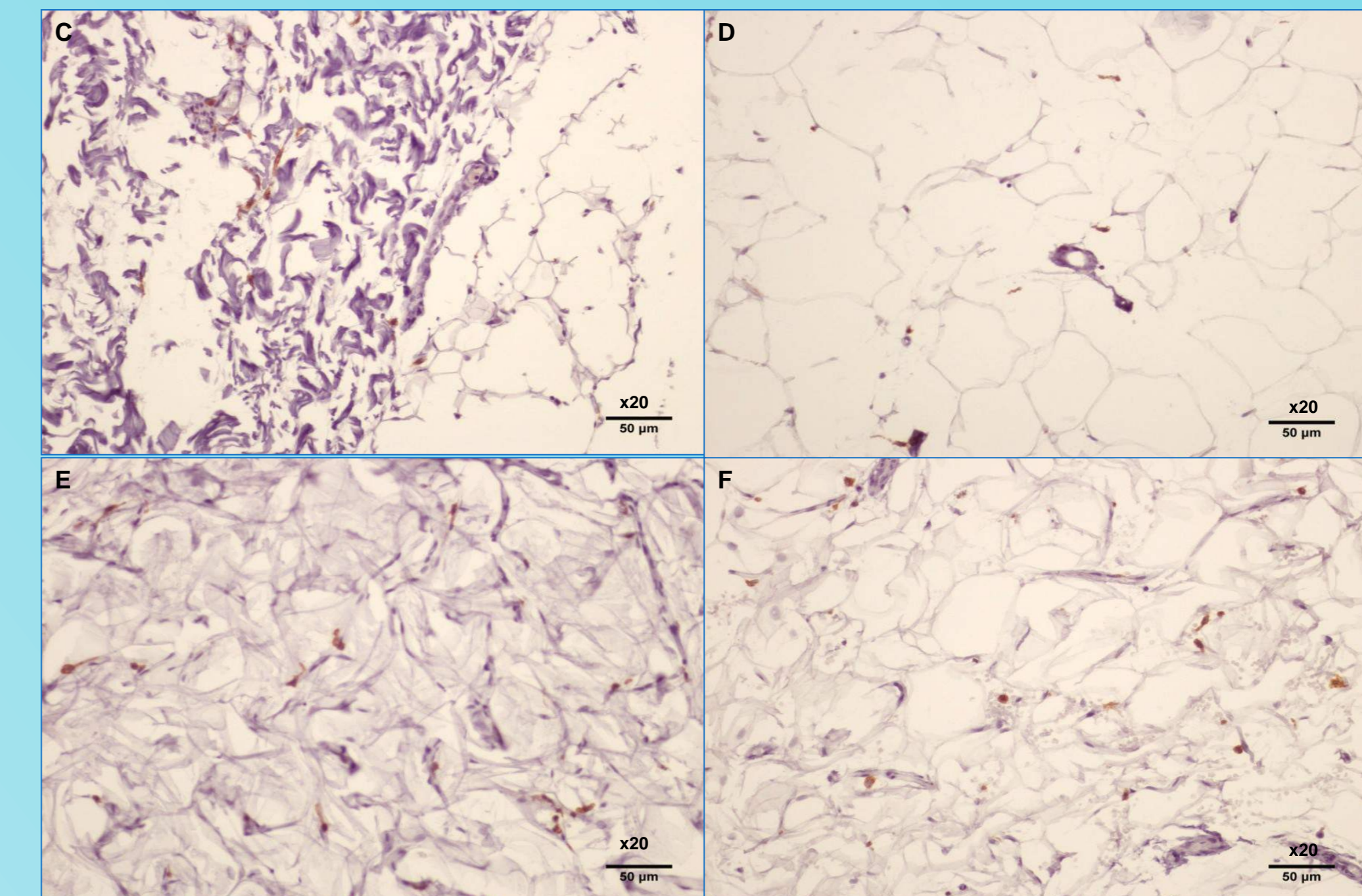


- CD163 (M2):** High intensity was observed in 33.3% of lean group A (A), 100% of lean (C) and obese group B (D), 71.4% of lean group C (E) and 50% of obese C (F) (p=0.012), whereas all of the obese A (B) showed low intensity. (brown = positive staining)

Table 4	CD163 Intensity	Group A		Group B		Group C		Total
		Lean	Obese	Lean	Obese	Lean	Obese	
	Low	66,7%	100,0%	0%	0%	28,6%	50,0%	38,5%
	High	33,3%	0%	100,0%	100,0%	71,4%	50,0%	61,5%
	Total	100,0%	100,0%	100,0%	100,0%	100,0%	100,0%	100,0%

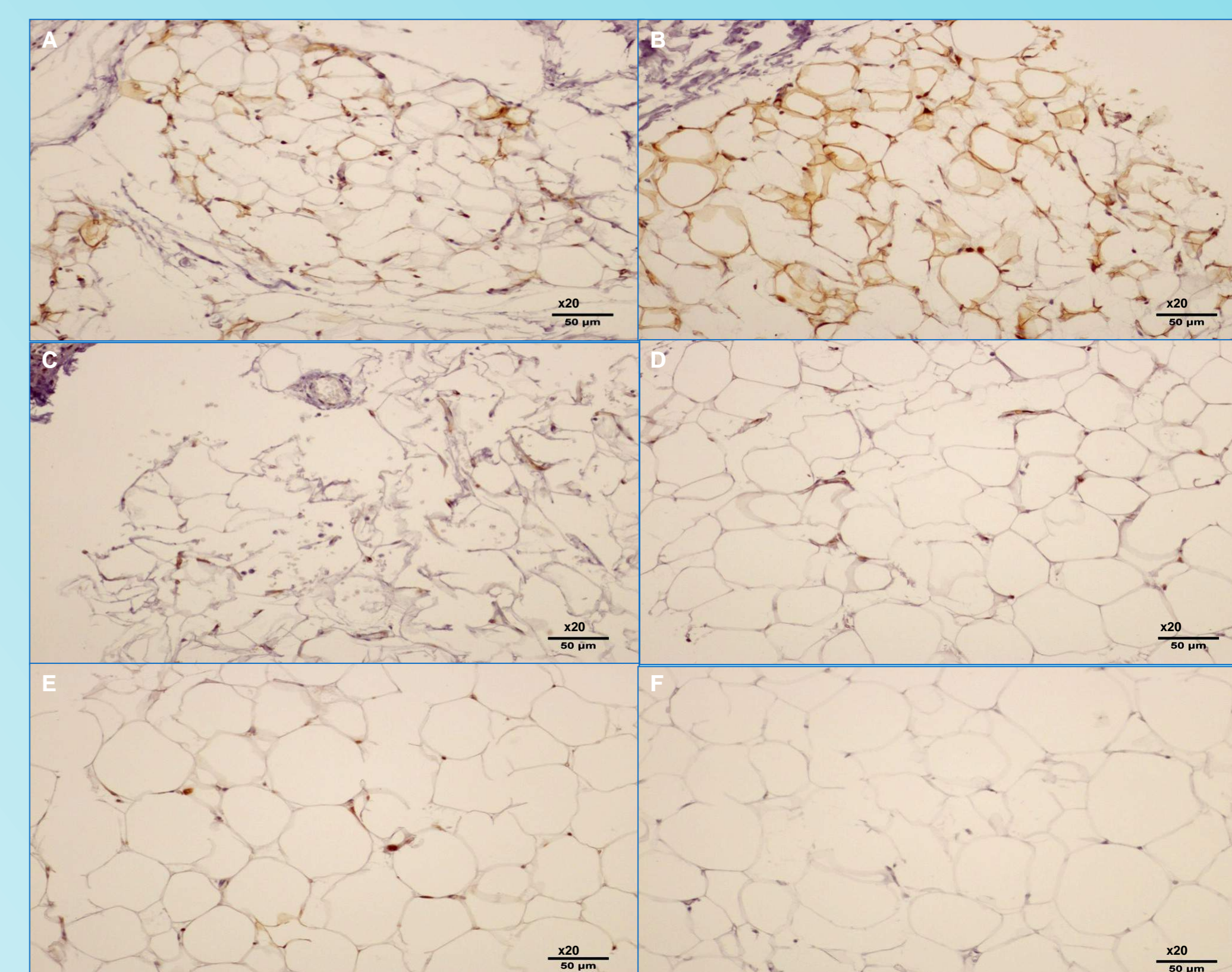


## Results



- CD40 (M1):** High intensity was observed in 50% of lean (A) and obese (B) group A, 70% of lean group B (C), 40% of obese group B (D) and 50% of lean group C (E) (p=0.015). (brown = positive staining)

Table 5	CD40 Intensity Macrophages	Group A		Group B		Group C		Total
		Lean	Obese	Lean	Obese	Lean	Obese	
	Low	50,0%	50,0%	30,0%	60,0%	50,0%	100,0%	60,8%
	High	50,0%	50,0%	70,0%	40,0%	50,0%	0%	39,2%
	Total	100,0%	100,0%	100,0%	100,0%	100,0%	100,0%	100,0%



- CD40 was also expressed on the adipocytes and was at high intensity only on the adipocytes of lean group A (A, 37.5%) and obese A (B, 33.3%), (p=0.012). (brown = positive staining)

Table 5	CD40 Intensity Adipocytes	Group A		Group B		Group C		Total
		Lean	Obese	Lean	Obese	Lean	Obese	
	Low	62,5%	66,7%	100,0%	100,0%	100,0%	100,0%	90,2%
	High	37,5%	33,3%	0%	0%	0%	0%	9,8%
	Total	100,0%	100,0%	100,0%	100,0%	100,0%	100,0%	100,0%

## Conclusion

- Our study confirms the presence of both M1 & M2 macrophages in adipocytes of children and adolescents.
- The high distribution of CD206<sup>+</sup> (M2) macrophages and high intensity of CD163 (M2) in the younger lean pre-pubertal children possibly reflects a strong anti-inflammatory profile in this young age group consistent with the high levels of HMW adiponectin<sup>3</sup>.
- The high intensity of CD40 (M1) in the younger obese pre-pubertal children, together with the lower distribution of CD206 (M2) and lower intensity of CD163 (M2), may put them at a higher risk for metabolic complications.
- The lack of CD40<sup>+</sup>(M1) macrophages in the obese adolescents alongside the positive staining for CD206 (M2) and CD163 (M2) may reflect a protective anti-inflammatory environment in these adolescents.

## References

- Olivia Osborn & Jerrold M Olefsky The cellular and signaling networks linking the immune system and metabolism in disease Nature Medicine, 18; 3, 363-374, 2012 .
- Esteve Ráfols M. Tejido adiposo: heterogeneidad celular y diversidad funcional. Endocrinol Nutr. 61; 100-112, 2014.
- Laura E. Hand et al. Adiponectin Induces A20 Expression in Adipose Tissue To Confer Metabolic Benefit Diabetes, epub Sept. 4 2014.