Elevated adipose tissue expression of interleukin-1 receptor-associated kinase (IRAK)-1 is linked with inflammatory state in obesity and insulin resistance

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Background: Modern lifestyle has led to the reduced physical activity and increased consumption of energy-rich foods which are the root cause of the substantial increase in obesity worldwide. Obesity is known to induce a state of chronic low-grade inflammation in both humans and rodents in which the expanded adipose tissue functions as an active endocrine organ and secretes adipokines including proinflammatory cytokines/chemokines, hormones, and other mediators. The emerging role of TLR2/4 as immuno-metabolic receptors points to key involvement of TLR/IL-1R/MyD88 pathway in obesity/type-2 diabetes (T2D). IL-1R-associated kinase (IRAK)-1 is a critical adapter protein of this signaling pathway. The changes in adipose tissue expression of IRAK-1 in obesity/T2D remain unclear.

Aims: **1)** To identify the expression level of IRAK-1 in obesity; **2)** To investigate the association of IRAK1 with inflammatory cytokines and signaling proteins; **3)** To investigate the relationship of IRAK-1 with HOMA-IR; **4)** To identify the impact of leptin and LPS on the regulation of IRAK-1.

Methods: A total of 49 non-diabetic (22 obese, 19 overweight and 8 lean), and 42 T2D (31 obese, 9 overweight and 2 lean), individuals were recruited in the study. Blood samples were collected and biochemical tests were performed. Human adipose tissue samples were collected by abdominal subcutaneous fat pad biopsy and stored at -80°C until use. The clinico-demographic data of the study participants are summarized in **Table 1**.

Real-time reverse-transcription polymerase chain reaction (RT-PCR): Total RNA was extracted from adipose tissues using RNeasy kit (Qiagen) and converted to cDNA. Expression level of IRAK1, TNF- α , IL-6, IL-18, CD68, CD163 and CD11c were then determined using gene-specific TaqMan primer Assays.

Immunohistochemistry: FFPE adipose tissue sections were incubated overnight with anti IRAK1 antibody using standard IHC protocol. Digital photomicrographs were taken and analyzed using ImageScope software

Confocal microscopy: FFPE adipose tissue samples were incubated overnight with anti IRAK1 antibody followed by a fluorescent secondary antibody conjugated with Alexa Fluor 594. Nucleus was stained with DAPI. Image processing and analysis was obtained by inverted Zeiss LSM710 Spectral confocal microscope.

In-vitro study: Experiments on Leptin induced adaptor proteins expression were performed on PBMC's of healthy Blood donors and expression level of adaptor proteins (IRAK1, MyD88, TOLLIP, TRAF6, TIRAP) were then determined using semi-quantitative RT-PCR using gene specific primers.

ELISA: Plasma levels of CCL5, C-reactive protein and adiponectin were measured using ELISA kits according to manufacturer's instructions.

Statistical Analysis: Unpaired Student t-test and Pearson's correlation were used for statistical analysis using GraphPad Prism software.

Table 1: Parameter	Non-diabetic			Diabetic		
	Lean	Overweight	Obese	Lean	Overweight	Obese
Total number	8	19	22	2	9	31
Male	2	12	11	1	5	18
Female	6	7	11	1	4	13
Age (Yrs.)	28-53	29-71	26-66	48-58	45-59	23-72
Body mass index (kg/m ²)	23.04 $\pm$ 2.33	28.31 $\pm$ 1.08	34.38 $\pm$ 2.78	25.47 $\pm$ 0.34	28.13 $\pm$ 1.02	33.76 $\pm$ 2.56
Fat percentage (fat %)	29.78 $\pm$ 6.19	32.65 $\pm$ 4.99	39.01 $\pm$ 4.35	32.10 $\pm$ 6.22	33.05 $\pm$ 5.38	37.33 $\pm$ 5.04
Glucose (mmol/L)	5.09 $\pm$ 0.64	5.60 $\pm$ 1.56	5.42 $\pm$ 0.79	5.80 $\pm$ 0.42	8.58 $\pm$ 1.77	8.64 $\pm$ 2.93
Cholesterol (mmol/L)	5.31 $\pm$ 1.25	4.89 $\pm$ 0.77	5.23 $\pm$ 1.07	5.40 $\pm$ 2.55	4.82 $\pm$ 1.69	5.07 $\pm$ 1.24
HDL (mmol/L)	1.79 $\pm$ 0.48	1.22 $\pm$ 0.23	1.12 $\pm$ 0.24	1.14 $\pm$ 0.15	1.15 $\pm$ 0.415	1.13 $\pm$ 0.29
LDL (mmol/L)	3.25 $\pm$ 1.05	3.18 $\pm$ 0.69	3.43 $\pm$ 0.94	3.70 $\pm$ 2.12	2.77 $\pm$ 1.39	3.01 $\pm$ 1.11
Triglycerides (mmol/L)	0.57 $\pm$ 0.22	1.22 $\pm$ 0.64	1.47 $\pm$ 0.82	1.25 $\pm$ 1.25	1.97 $\pm$ 0.88	1.91 $\pm$ 1.49
HbA1c (%)	5.69 $\pm$ 0.46	5.92 $\pm$ 1.65	5.86 $\pm$ 0.49	6.00 $\pm$ 0.28	7.54 $\pm$ 1.67	8.24 $\pm$ 1.51

Results: In non-diabetics, IRAK-1 gene expression was elevated in obese ($P = 0.01$) and overweight ($P = 0.04$) as compared with lean individuals and this increase correlated with body mass index ($r = 0.45$; $P = 0.001$) and fat percentage ($r = 0.36$; $P = 0.01$). In diabetics, IRAK-1 mRNA expression was also higher in obese as compared with lean subjects ($P = 0.012$) (Fig :1). As also shown by immunohistochemistry/confocal microscopy in non-diabetics and by immunohistochemistry in diabetics, IRAK-1 protein expression was higher in obese than overweight and lean adipose tissues (Fig: 2). IRAK-1 gene expression correlated positively/significantly with mRNAs of TNF- α ($r = 0.46$; $P = 0.0008$), IL-6 ($r = 0.30$; $P = 0.03$) and IL-18 ($r = 0.31$; $P = 0.028$) in non-diabetics; and only with TNF- α ($r = 0.32$; $P = 0.03$) in diabetics (Fig: 3). IRAK-1 expression also correlated positively/significantly with CD68 ($r = 0.32$; $P = 0.02$), CD11c ($r = 0.30$; $P = 0.03$), and CD163 ($r = 0.43$; $P = 0.001$) in non-diabetics; and only with CD163 ($r = 0.34$; $P = 0.02$) in diabetics (Fig: 4). IRAK-1 mRNA levels also correlated with plasma markers including CCL-5 ($r = 0.39$; $P = 0.02$), C-reactive protein ($r = 0.48$; $P = 0.005$) and adiponectin ($r = -0.36$; $P = 0.04$) in non-diabetics; but not in diabetics (Fig: 5). IRAK-1 expression related with TLR2 ($r = 0.39$; $P = 0.007$) and MyD88 ($r = 0.36$; $P = 0.01$) in non-diabetics; and MyD88 ($r = 0.52$; $P = 0.0003$) in diabetics (Fig: 6). Our data shows that IRAK-1 is a critical regulator of inflammation. Interestingly, we found that HOMA-IR was correlated positively with IRAK-1 ($r=0.38$; $P=0.02$) in non-diabetics (Fig: 7). In-vitro experiments with PBMC of healthy donors show Leptin induces the expression of IRAK1 and other adaptor proteins (Fig: 8).

Fig1: Adipose tissue IRAK-1 expression is significantly elevated in obese individuals with or without T2D

Fig 2: Adipose tissue IRAK-1 protein expression is significantly elevated in obese individuals with or without T2D

Fig 3: Increased adipose tissue expression of IRAK-1 mRNA is concordant with local expression of proinflammatory mediators

Fig 4 : Enhanced IRAK-1 gene expression correlates with adipose tissue infiltration by monocytes/macrophages

Fig 5: Adipose tissue IRAK-1 mRNA expression correlates with systemic inflammatory or obesity clinical markers

Fig 6: IRAK-1 gene expression in the adipose tissue correlates with TLR2 and/or MyD88 gene expression

Fig7: Increased adipose tissue expression of IRAK-1 mRNA is correlated with HOMA-IR.

Fig 8: Leptin upregulates IRAK-1 and other adaptor protein gene expression in PBMC

Conclusion: Taken together, our data show that adipose tissue IRAK-1 expression was significantly upregulated in obese and overweight individuals as compared with lean. Based on consensus with both circulatory and local inflammatory signatures, IRAK-1 expression may be regarded as an additional tissue marker for meta-inflammation in obesity. The study has clinical significance as the approaches intending IRAK-1 suppression may improve metabolic complications in Obesity and T2D.

Acknowledgment: Kuwait Foundation for the Advancement of Sciences(KFAS) (Grant # RA2010-003)