Do the STAP Test --- Prevent the Diabetes

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Diagnosis of Diabetes

Fasting Plasma Glucose
(Fasting Blood Sugar)

Normal : 80 - 99 mg/dl

Pre-diabetes : 100 - 125 mg/dl

Diabetes : ≥126 mg/dl

Hemoglobin A1C (HbA1C): ≥ 6.5%

Global Statistics on Diabetes

Prevalence

Yr. 2021: 10%; Total: 537 million

Projected in 2030: 643 million

Projected in 2045: 784 million

Currently undiagnosed DM patients: 240 million

Death

6.7 million

Cost

966 billion US dollars

(Ref: IDF Atlas 2021)

US Statistics on Diabetes

Prevalence:	In 2019:	37.3 million (11.3%)
Death:	In 2019:	283,000
Cost:	In 2018:	327 billion dollars

(Ref: American Diabetes Association)

Background

Animal Study

2007: *Cani et al., Metabolic endotoxemia initiates obesity and insulin resistance. Diabetes. 2007 Jul;56(7):1761-72.*

We know: Insulin resistance leads to hyperglycemia followed by diabetes

Metabolic Endotoxemia

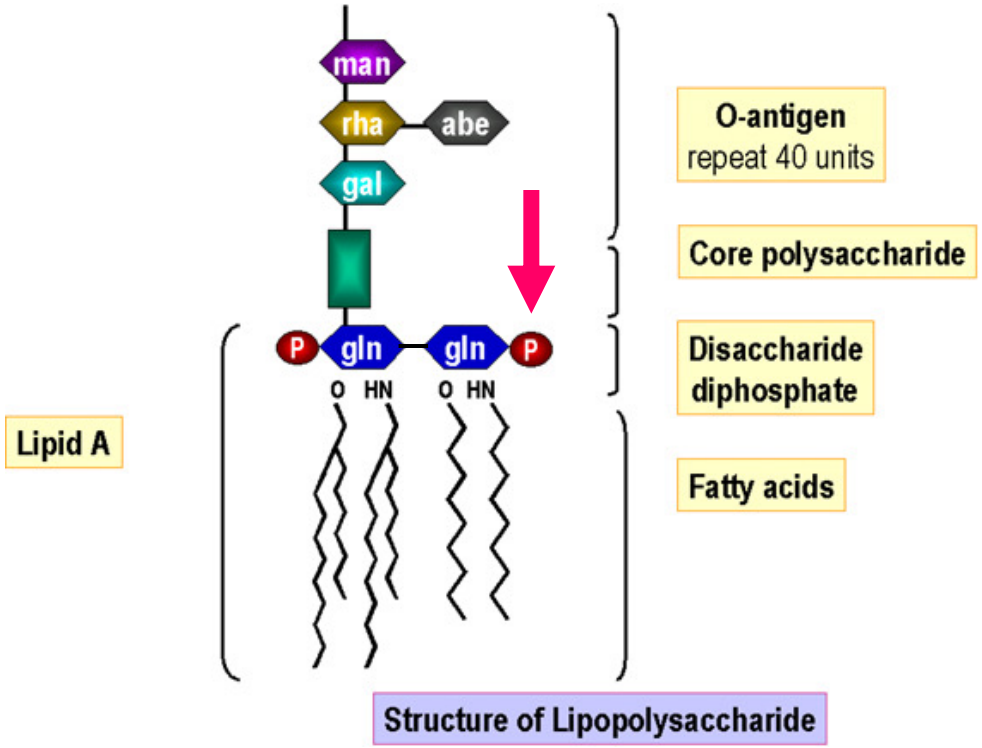
Definition

2-3 fold persistent increase in circulating endotoxin concentrations compared to the normal levels

(Metabolic Endotoemia: 4-12 EU/ml serum)

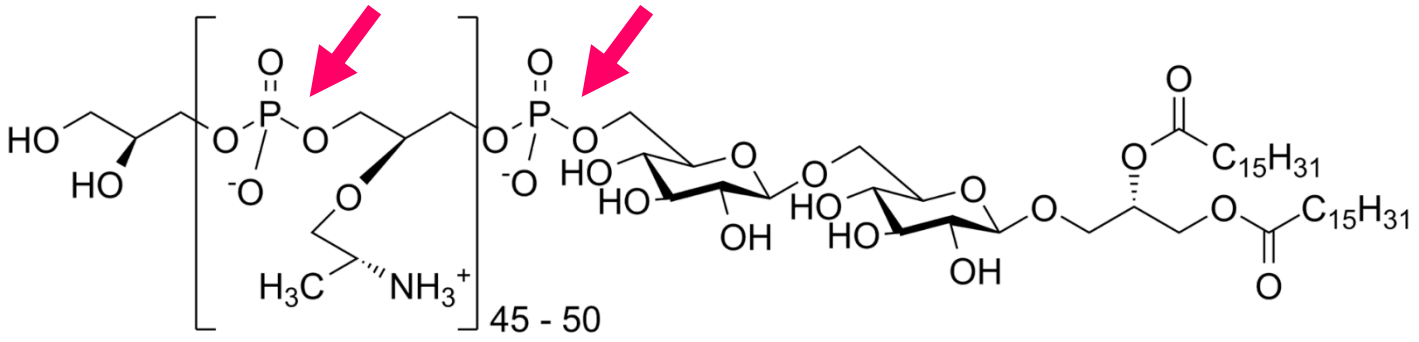
Gram-Negative Endotoxin

Gram-negative bacterial cell wall component lipopolysaccharides (LPS)



Gram-Positive Endotoxin

Gram-positive bacterial cell wall component
lipoteichoic acids (LTA)



Metabolic Endotoxemia

Caused By

High-fat diet

High-fructose diet

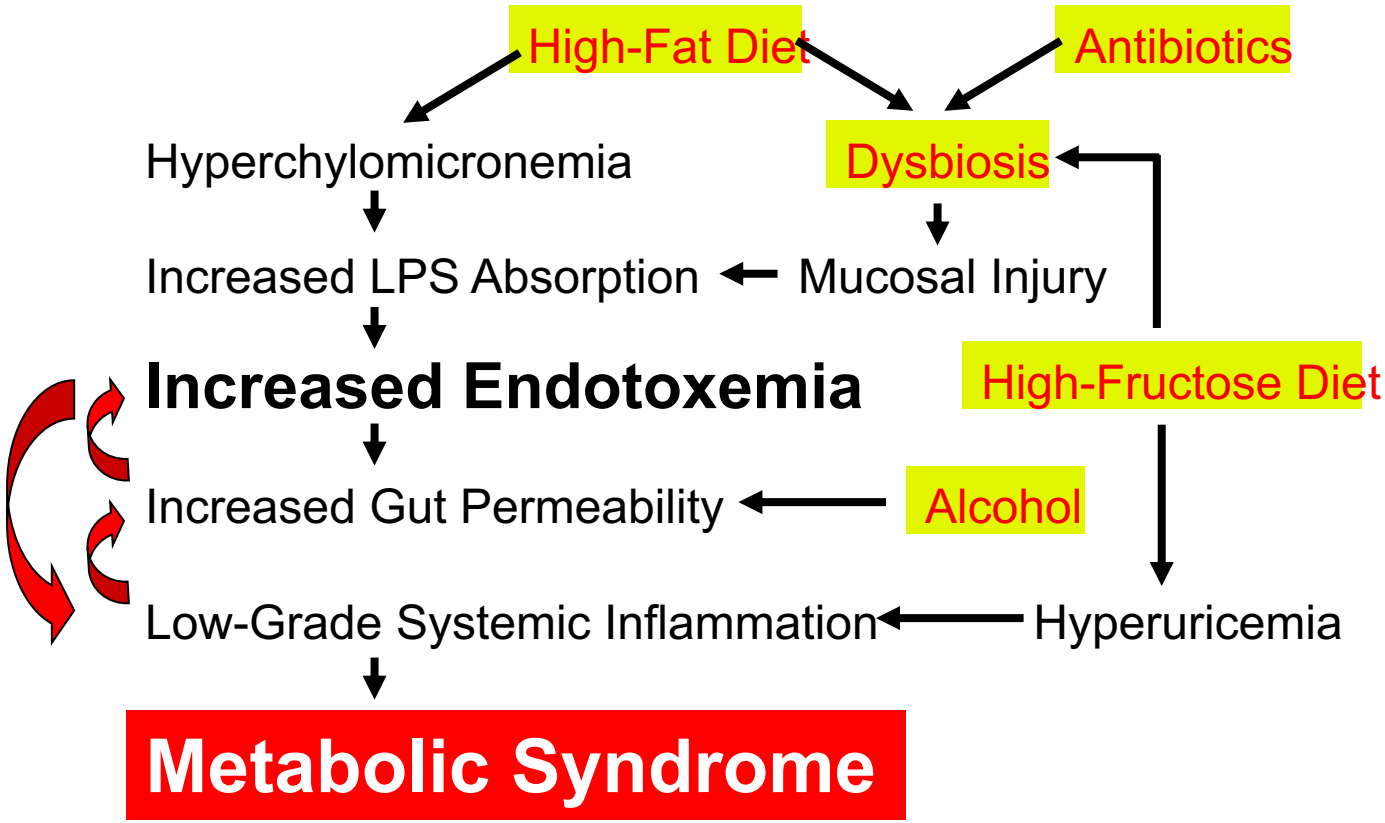
Alcohol (wine)

Antibiotics

Dysbiosis (abnormal gut flora)

The Metabolic Syndrome

Mechanisms



Chronic Inflammation causes Diabetes

- Nordmann et al., The Role of Inflammation in β-cell Dedifferentiation. Sci Rep. 2017 (https://www.ncbi.nlm.nih.gov/pmc/articles/PMC5524956/#CR7).

- Larsen et al., Interleukin-1-receptor antagonist in type 2 diabetes mellitus. N Engl J Med. 2007 (https://pubmed.ncbi.nlm.nih.gov/17429083/).

- Hotamisligil GS, Shargill NS, Spiegelman BM. Adipose expression of tumor necrosis factor-alpha: direct role in obesity-linked insulin resistance. Science. 1993 (https://pubmed.ncbi.nlm.nih.gov/7678183/).

Intestinal Alkaline Phosphatase (IAP)

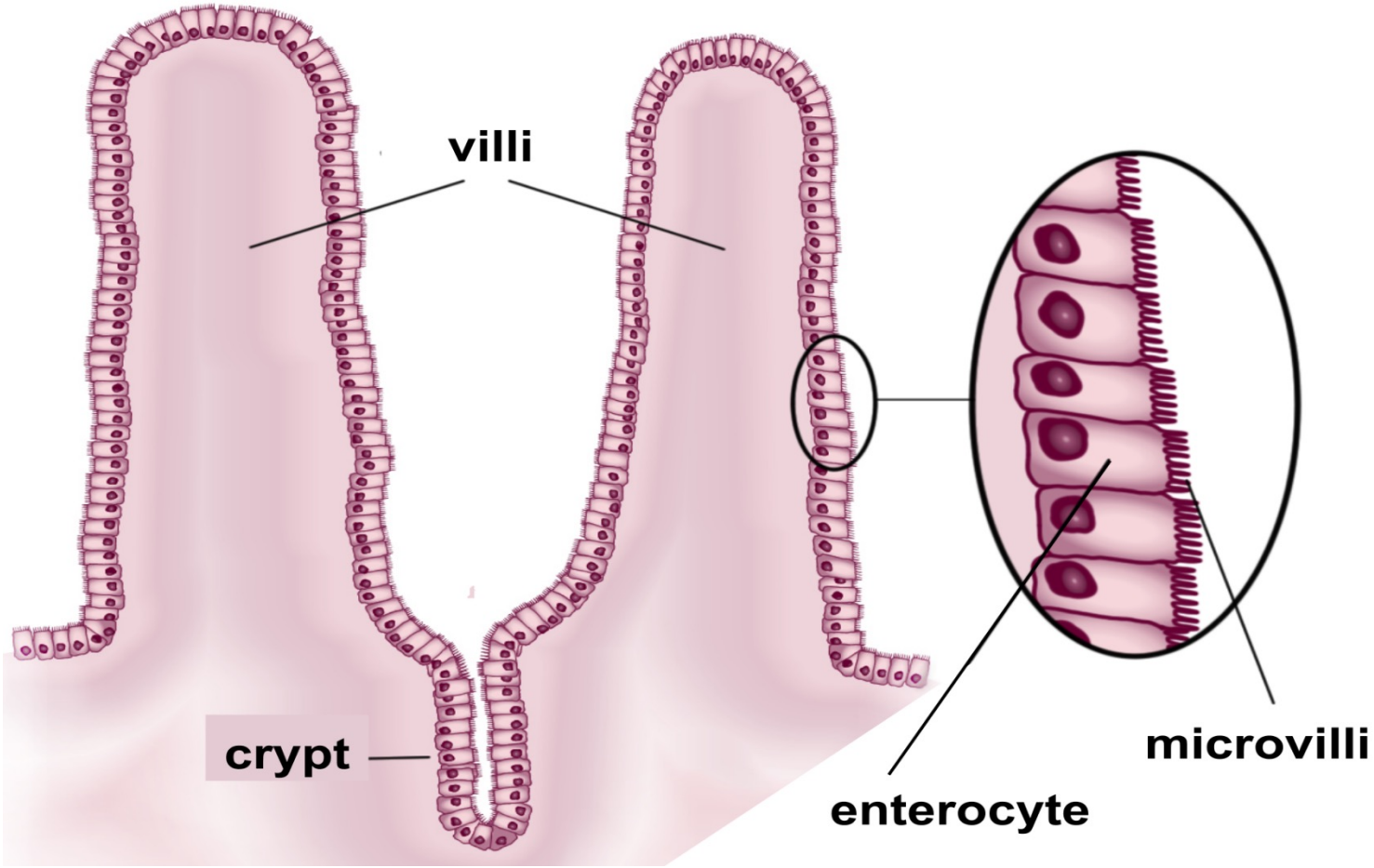
Alternatively known as

Stool Alkaline Phosphatase (STAP)

What is IAP?

- A 150 Kd (528 aa, dimeric) protein (enzyme) secreted by villus-associated enterocyte cells lining the small intestine, and excreted with stool
- Human IAP gene (ALP1) is located in the long-arm of chromosome 2

Small Intestinal Lumen

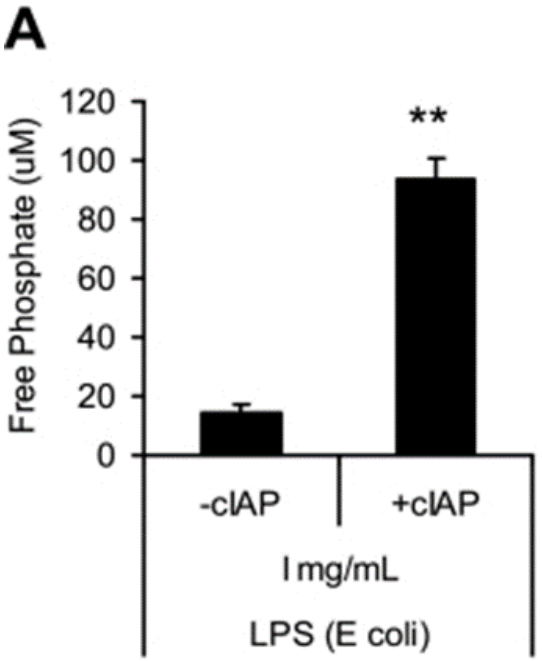


Small Intestinal Wall

Function of IAP

- IAP functions efficiently at alkaline pH (7-10)
- **It detoxifies bacterial toxins, e.g., endotoxins lipopolysaccharides (LPS) and lipoteichoic acids (LTA) by dephosphorylation**
- Also detoxifies CpG DNA,, ATP, Uridine, flagellin, etc.
- Maintains homeostasis of intestinal microbiota
- Limits fat absorption

Calf IAP (cIAP) Detoxifies LPS



Chen, Malo, -- Hodin: Identification of specific targets for the gut mucosal defense factor intestinal alkaline phosphatase. Am J Physiol Gastrointest Liver Physiol. 2010 Aug;299(2):G467-75.

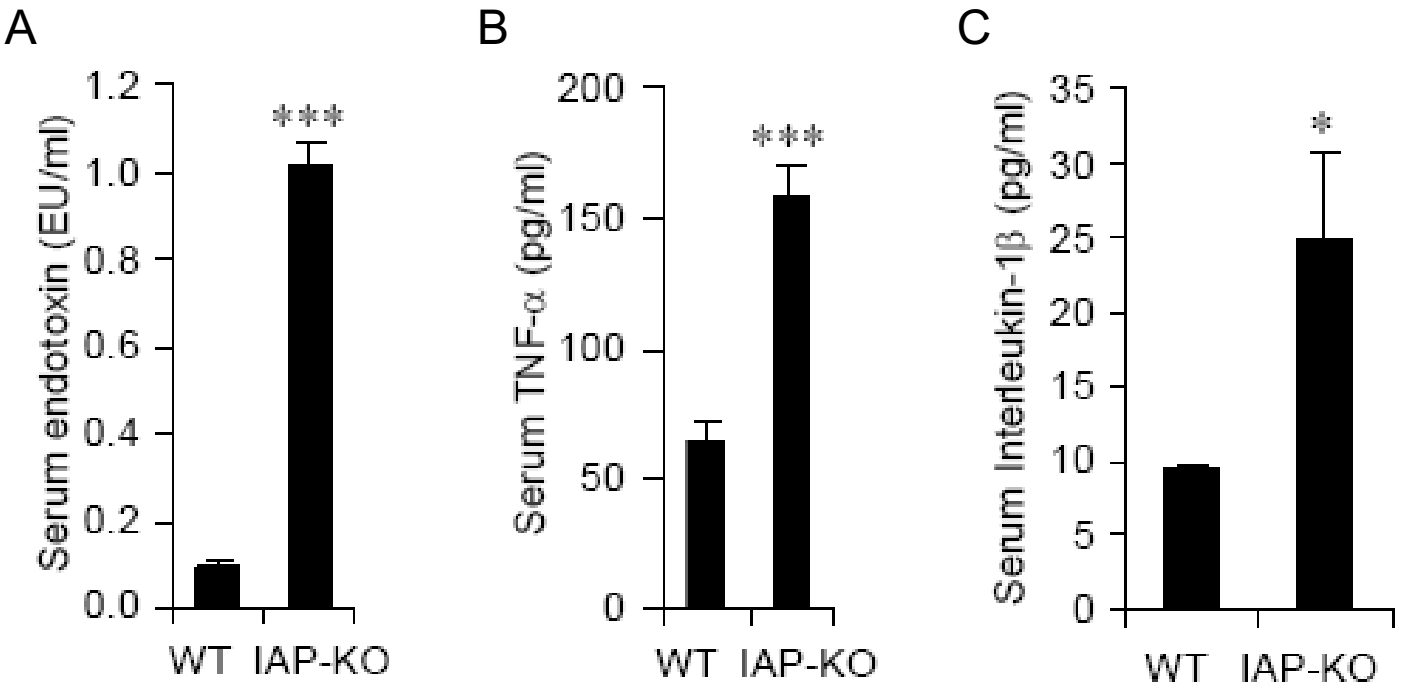
Hypothesis

Mice deficient in IAP (IAP gene knockout mice) should develop hyperglycemia (diabetes)

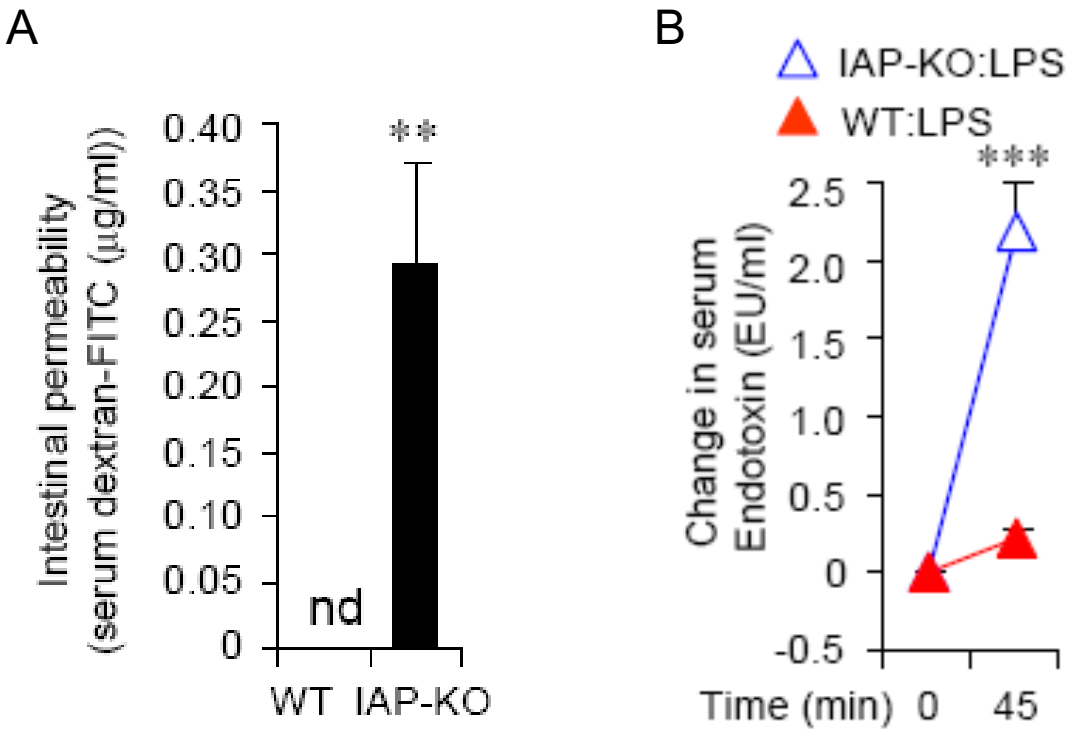
2013: *Kaliannan et al. Intestinal alkaline phosphatase prevents metabolic syndrome in mice. Proc Natl Acad Sci U S A 2013;110:7003–8.*

Metabolic Syndrome: Hyperglycemia, Hypertension, Dyslipidemia, Obesity

IAP Knockout (KO) Mice Suffer from Metabolic Endotoxemia and Systemic Inflammation

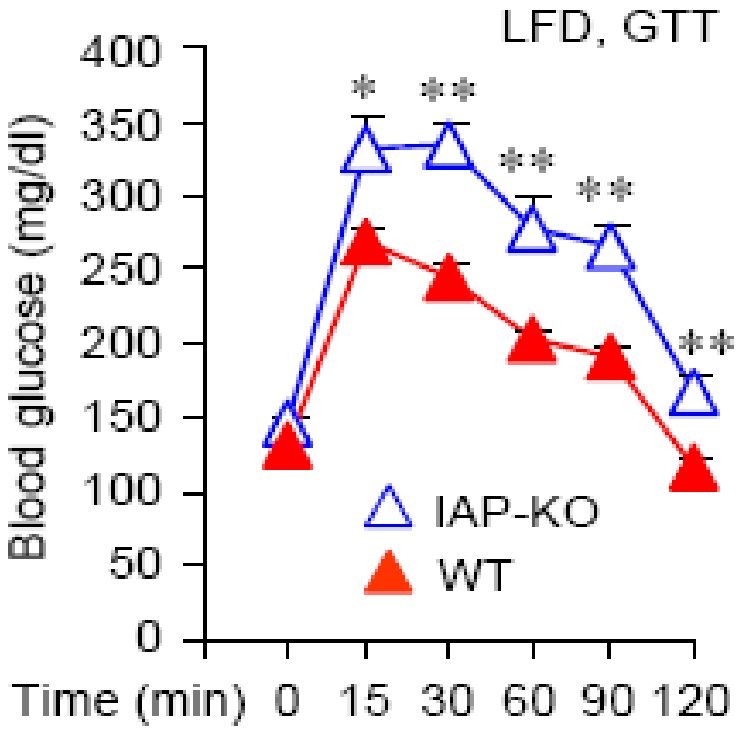


IAP-KO Mice Show Increased Gut Permeability



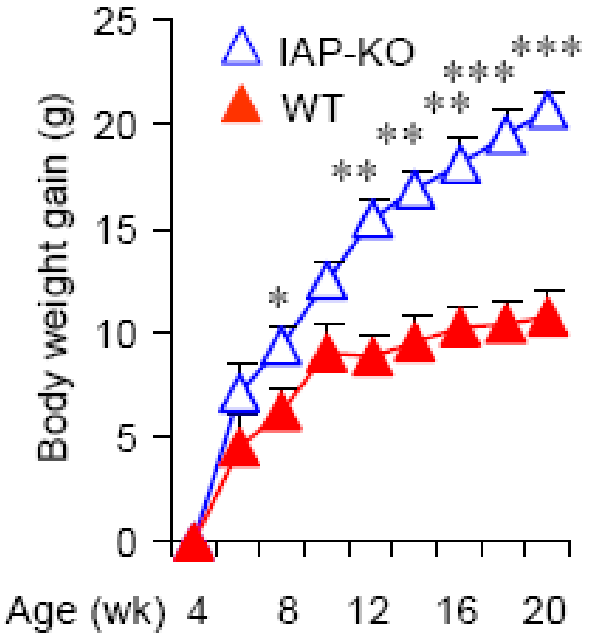
IAP-KO Mice Suffer from Glucose Intolerance

A



IAP-KO Mice Are Obese

A



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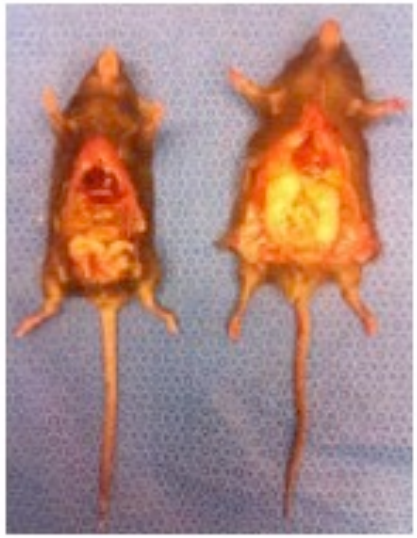
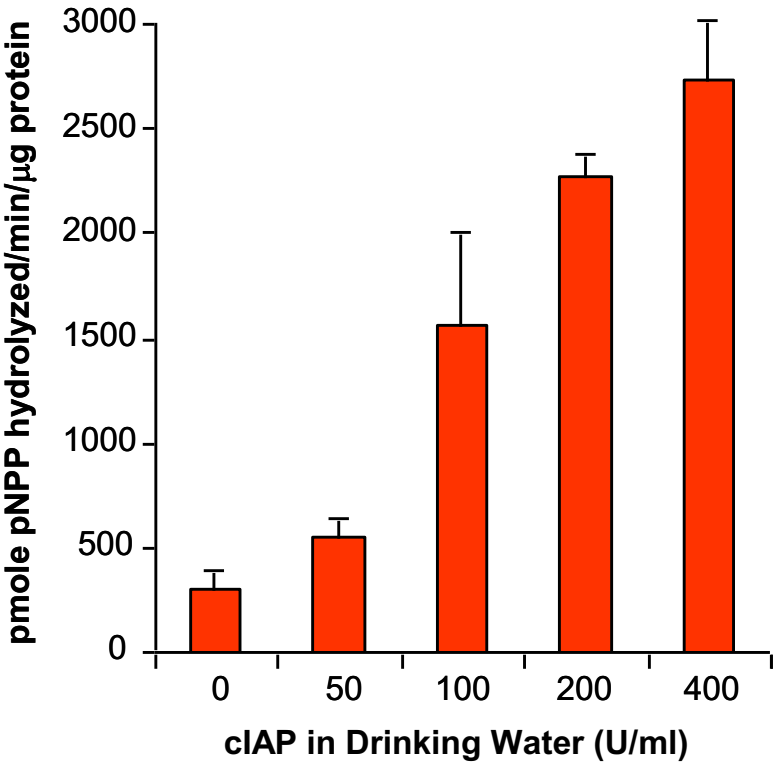
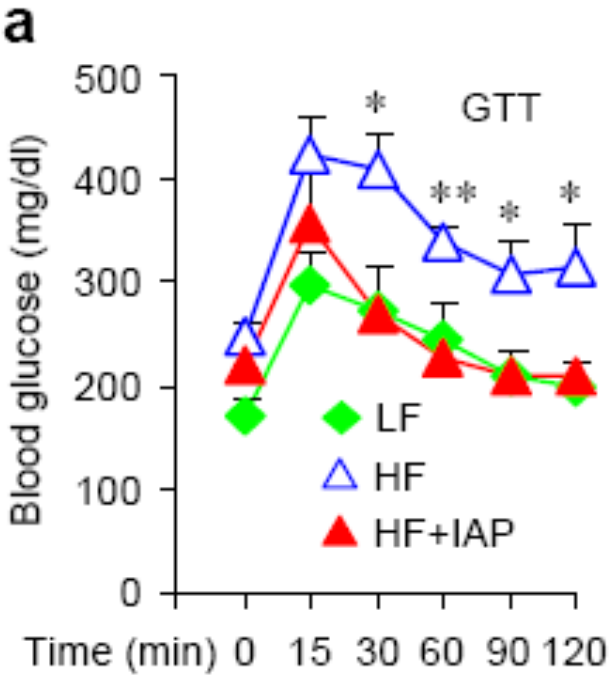


WT IAP-KO

IAP Supplementation with Drinking Water Increases Stool IAP Activity

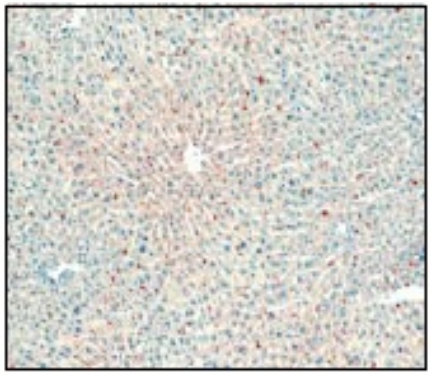


Oral IAP Supplementation Prevents High-Fat Diet-Induced Metabolic Syndrome (Glucose Intolerance)



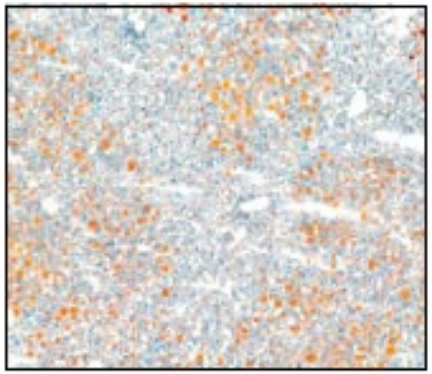
IAP Prevents Liver Steatosis

Oil Red O staining



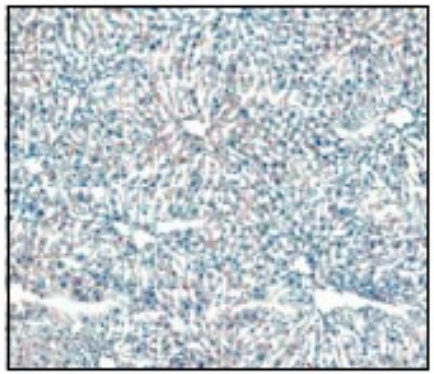
LF

Oil Red O staining



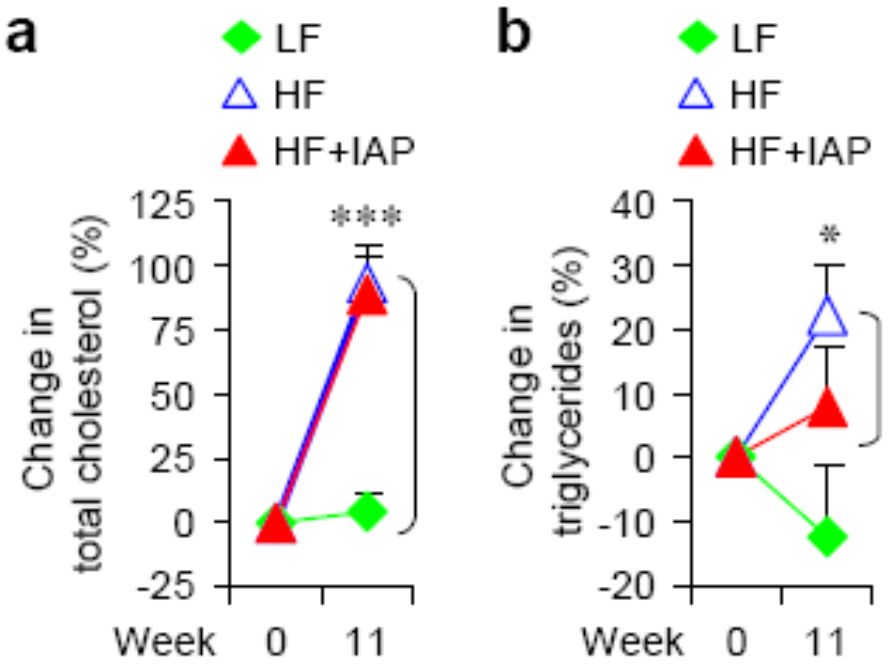
HF

Oil Red O staining

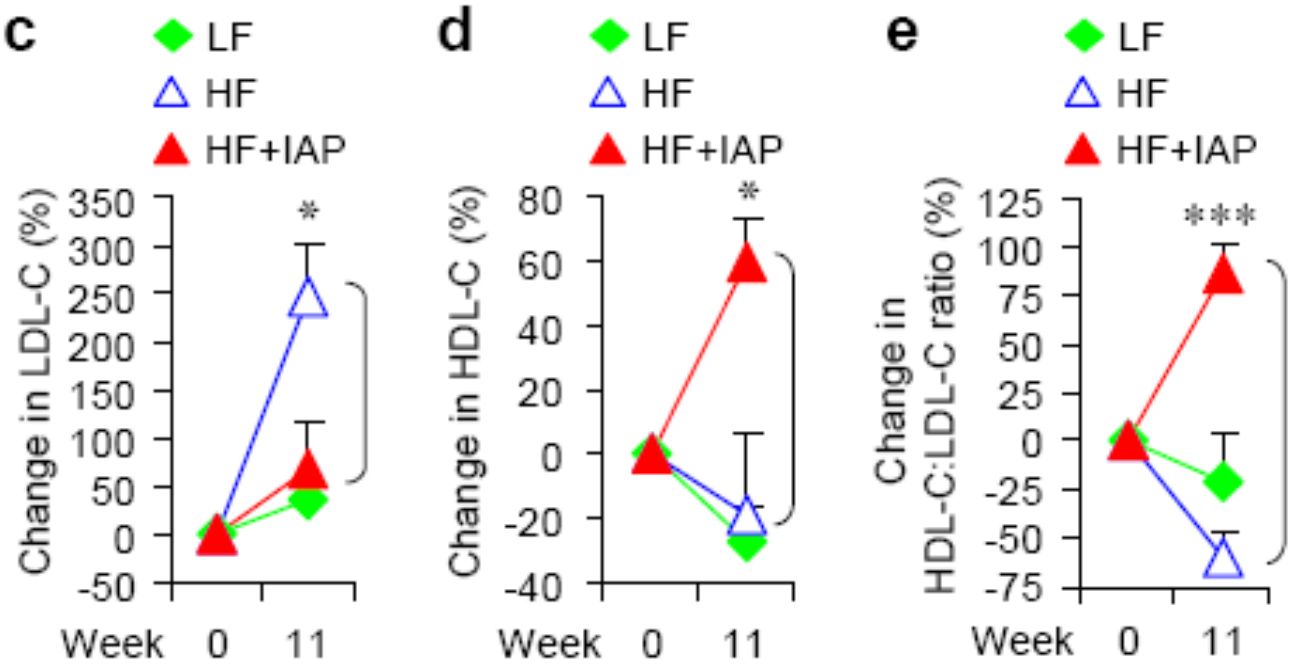


HF+IAP

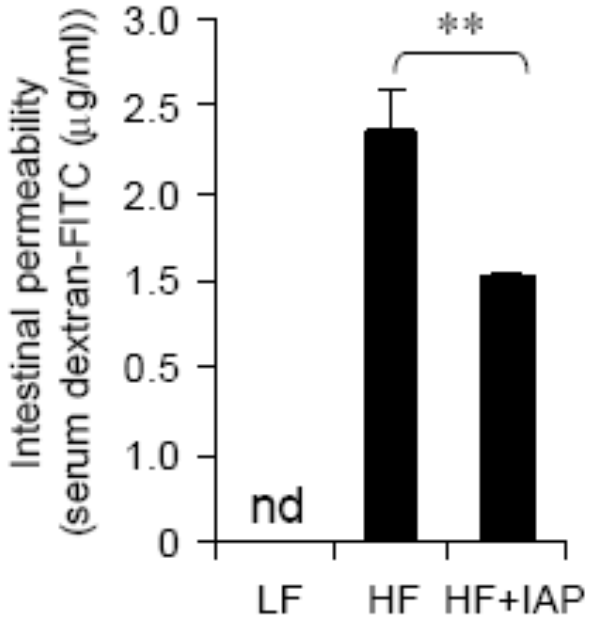
IAP Prevents HFD-Induced Dyslipidemia



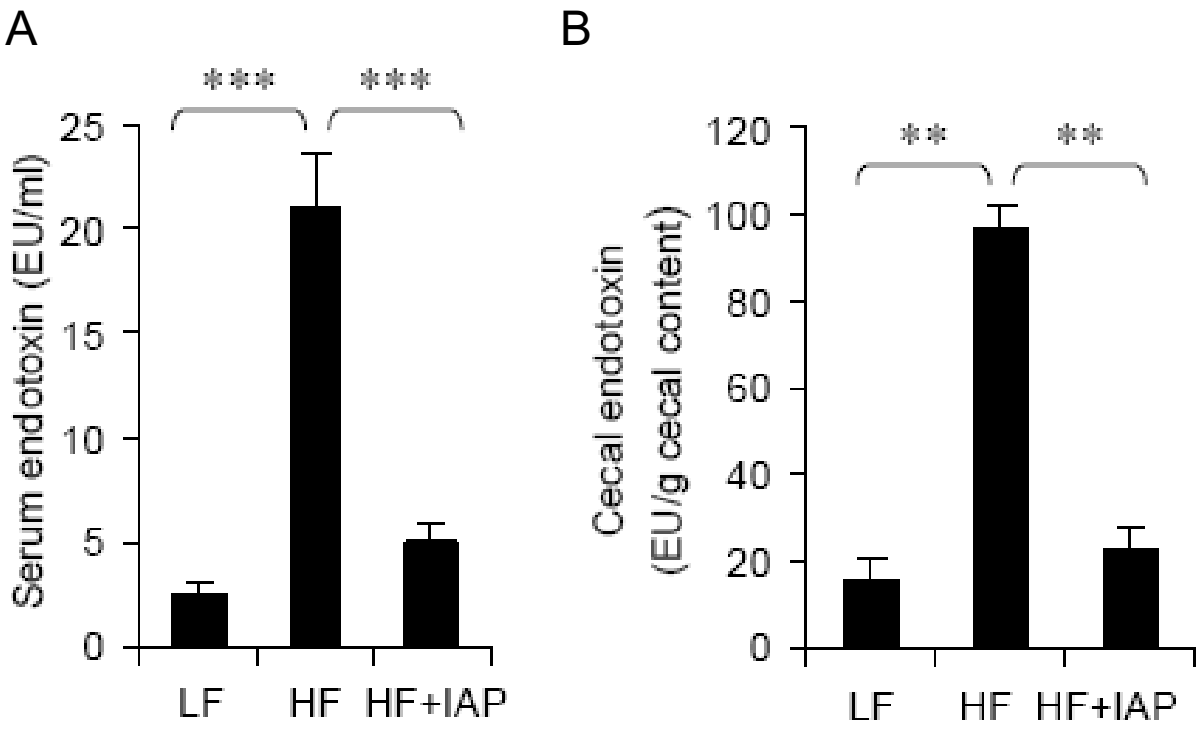
IAP Prevents HFD-Induced Dyslipidemia



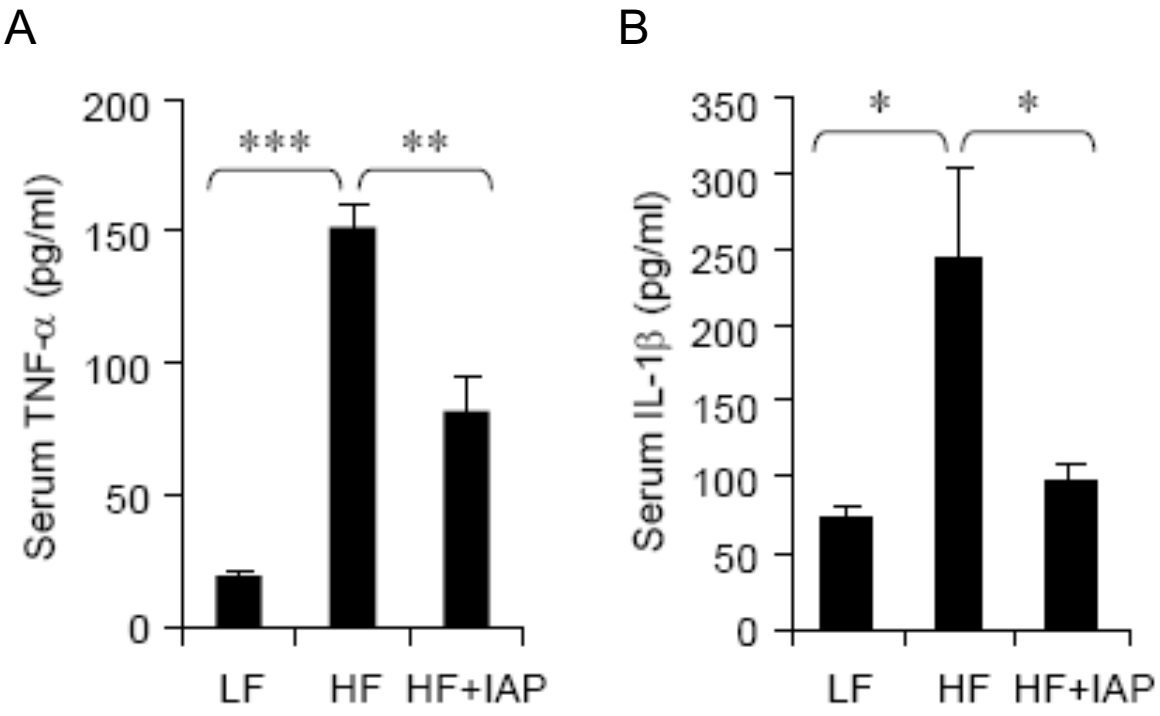
IAP Prevents HFD-Induced Gut Permeability



IAP Prevents HFD-Induced Endotoxemia



IAP-Treated MetS^{+} Mice Have Less Systemic Inflammation



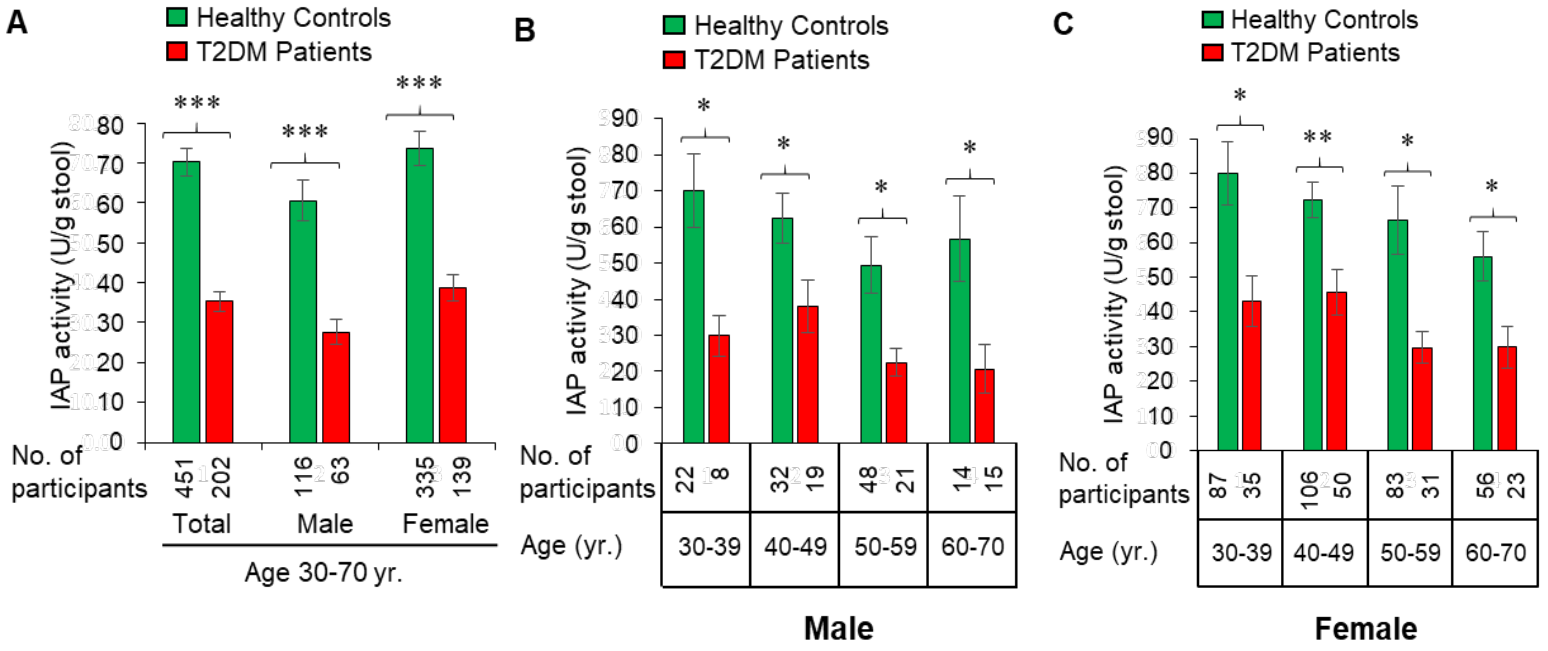
Human Study

Hypothesis

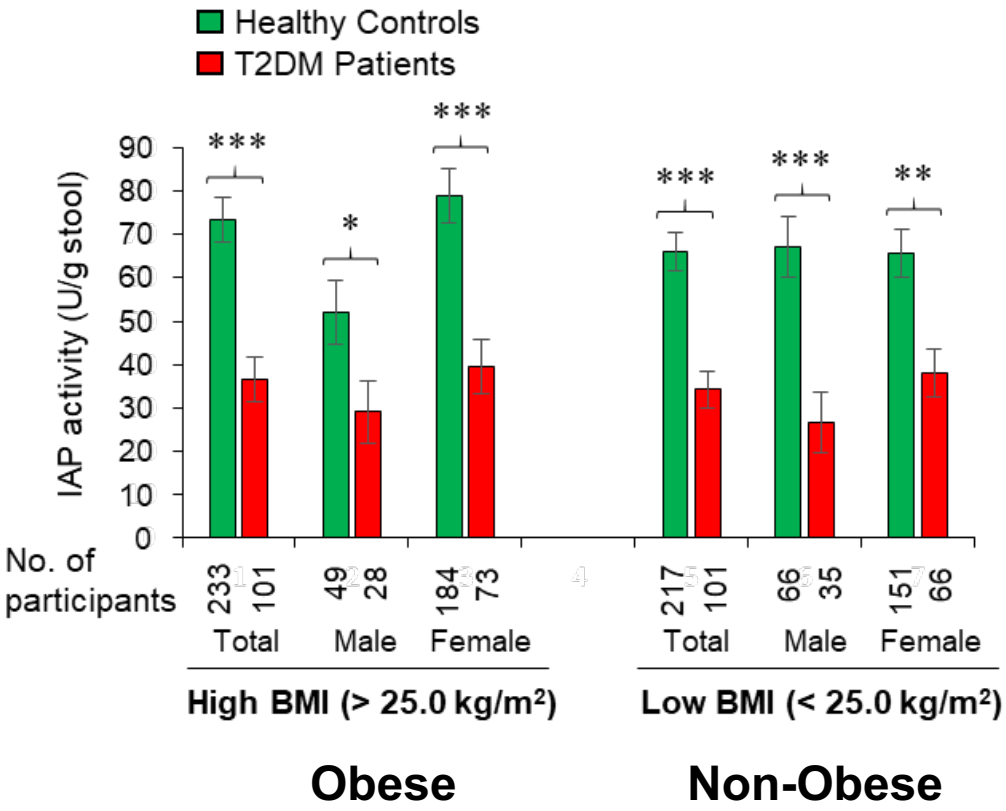
If IAP knockout mice suffer from hyperglycemia (diabetes) then humans with diabetes should have less amount of IAP in their stool

2015: *Malo MS. A high level of intestinal alkaline phosphatase is protective against type 2 diabetes mellitus irrespective of obesity. EBioMedicine 2015;2:2016–23.*

Patients with type 2 diabetes mellitus (T2DM) have low levels of intestinal alkaline phosphatase (IAP/STAP) in their stool



High levels of IAP is protective against diabetes irrespective of obesity (body mass index, BMI)



Human Study on a Prospective Cohort (2015-2020)

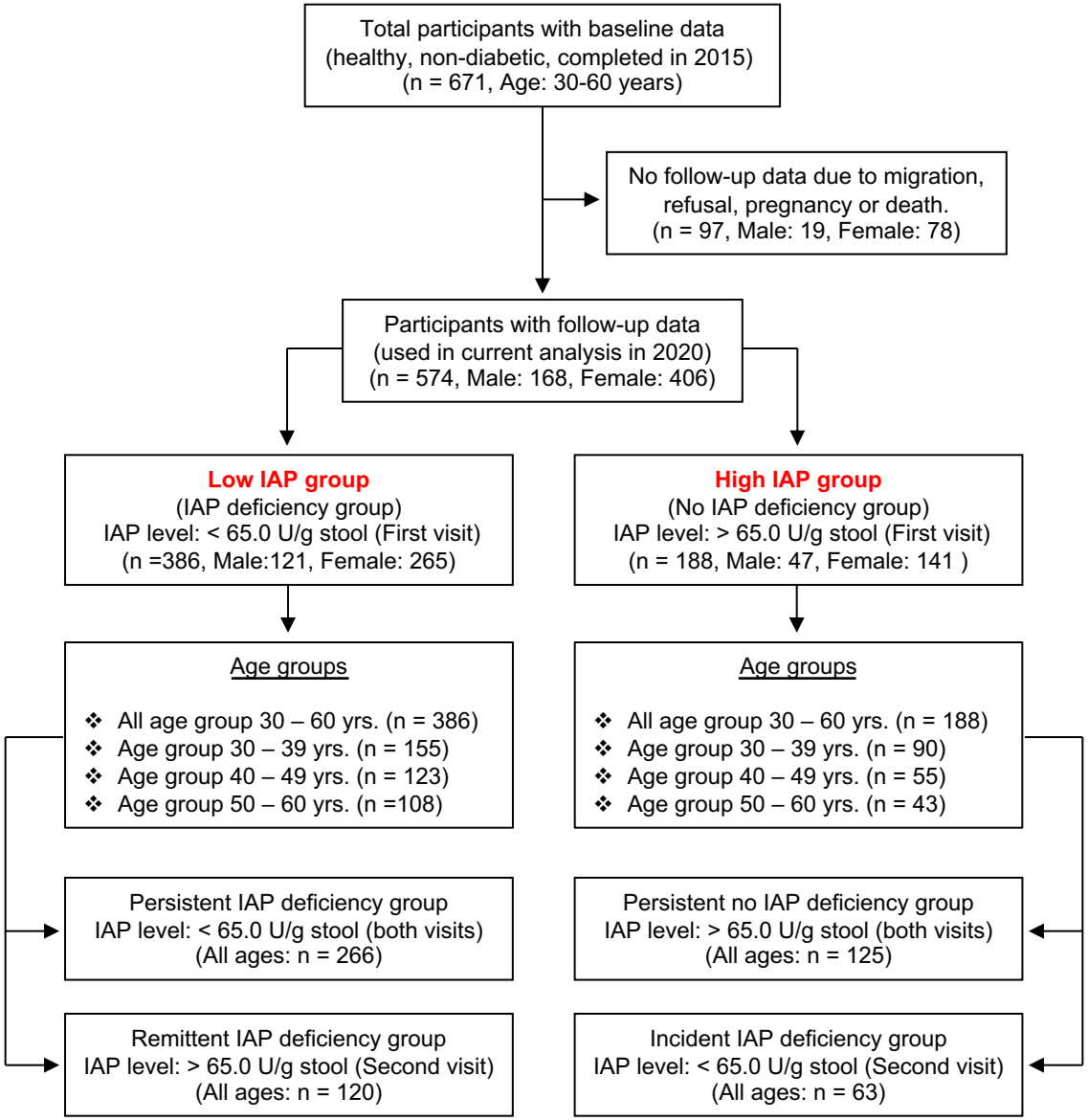
Hypothesis

Background:

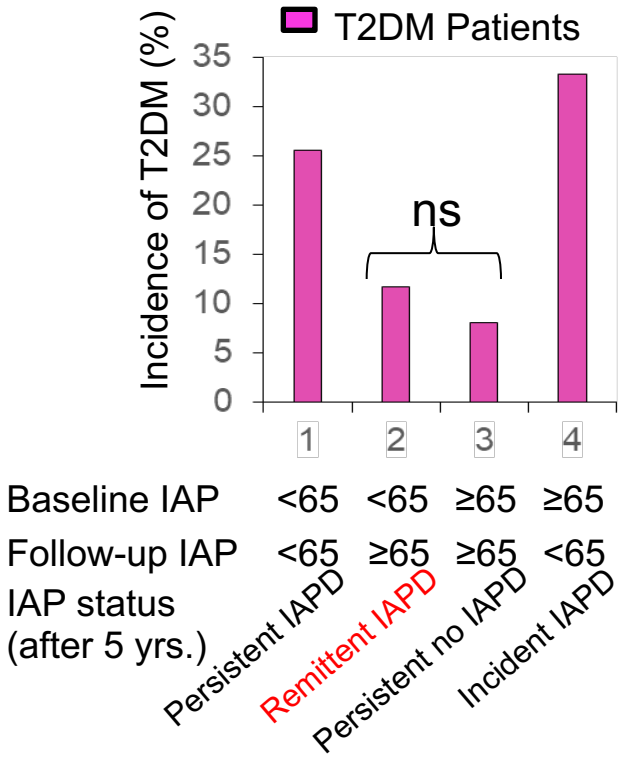
- **IAPD is associated with diabetes in humans**
- **IAPD causes Metabolic Syndrome in mice**

Hypothesis: IAPD causes diabetes

Research Design of the Prospective Cohort Study



IAPD is associated with a higher incidence of diabetes



Note: Remission of IAPD (**Remittent IAPD**) prevents diabetes
ns, not significant

Table 1. Intestinal alkaline phosphatase deficiency (IAPD) is associated with increased risk of developing type 2 diabetes mellitus (T2DM).

IAPD Status	Total Participants	T2DM N (%)	Relative Risk (95% Confidence Interval)				
			Model 1[a]	Model 2[b]	Model 3[c]	Model 4[d]	Model 5[e]
Persistent no IAPD	125	10 (8.0)	1.00	1.00	1.00	1.00	1.00
Incident IAPD	63	21 (33.3)	4.17 (2.09 - 8.30)***	3.78 (1.89 - 7.54)***	4.31 (2.00 - 9.27)***	4.17 (1.93 - 9.00)***	3.69 (1.76 - 7.71)***
Persistent IAPD	266	68 (25.6)	3.20 (1.70 - 5.99)***	3.01 (1.60 - 5.65)***	3.33 (1.65 - 6.71)***	3.27 (1.62 - 6.61)***	3.27 (1.64 - 6.50)***
Remittent IAPD	120	14 (11.7)	1.46 (0.67 - 3.16)	1.49 (0.69 - 3.21)	2.00 (0.88 - 4.56)	2.05 (0.89 - 4.68)	2.24 (0.99 - 5.11)

Legend:

a, *Model 1: Unadjusted model.*

b, *Model 2: Adjusted for age and sex at baseline (Visit 1).*

c, *Model 3: Adjusted for variables in Model 2 plus body mass index (BMI), systolic blood pressure and diastolic blood pressure at baseline (Visit 1).*

d, *Model 4: Adjusted for variables in Model 3 plus creatinine, cholesterol, HDL, LDL, triglycerides and ALT at baseline (Visit 1).*

e, *Model 5: Adjusted for variables in Model 4 plus fasting plasma glucose (FPG) at baseline (Visit 1).*

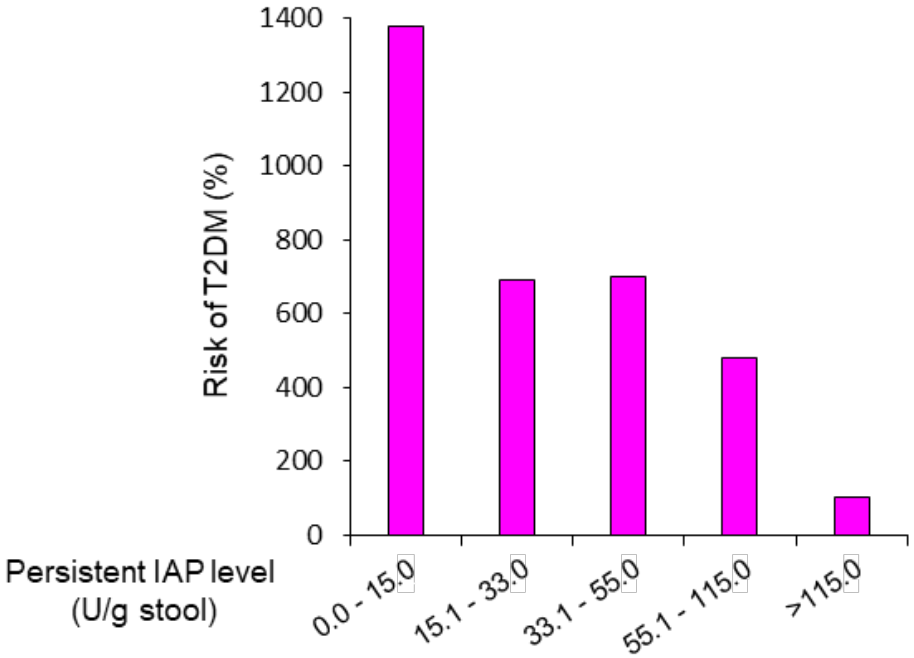
***, P <0.001.*

Table 2. The Lower the persistent IAP level the higher the rate of increase of fasting glycemia and relative risk of developing T2DM.

Pentile	Persistent IAP level (U/g stool)	No. of participants (T2DM, %)	Relative risk (95% confidence Interval)	First visit FPG level (mmol/l)/(mg/dl)	Second visit FPG level (mmol/l)/(mg/dl)	Percentage (%) increase of FPG level
Pentile 1	0 – 15.0	30 (14, **46.7%**)	**13.8 (1.87-101.3)****	4.4 ± 0.7 (79.2 ± 12.6)	6.9 ± 2.5*** (124.2 ± 45.0)	**34.1*****
Pentile 2	15.1 – 33.0	38 (8, 21.1%	6.9 (0.91-52.60)	4.5 ± 0.8 (81.0 ± 14.4)	6.1 ± 1.0*** (109.8 ± 18.0)	25.9*
Pentile 3	33.1 – 55.0	39 (9, 23.1%	7.0 (0.93-52.57)	4.3 ± 0.8 (77.4 ± 14.4)	6.0 ± 1.1*** (108.0 ± 19.8)	25.2**
Pentile 4	55.1 – 115.0	58 (8, 13.8%)	4.8 (0.63-36.71)	4.3 ± 0.8 (77.4 ± 14.4)	5.6 ± 1.0*** (100.8 ± 18.0)	23.9*
Pentile 5	>115.0	34 (1, **2.9%**)	1.0	4.5 ± 0.7 (81.0 ± 12.6)	5.5 ± 0.7*** (99.0 ± 12.6)	17.3

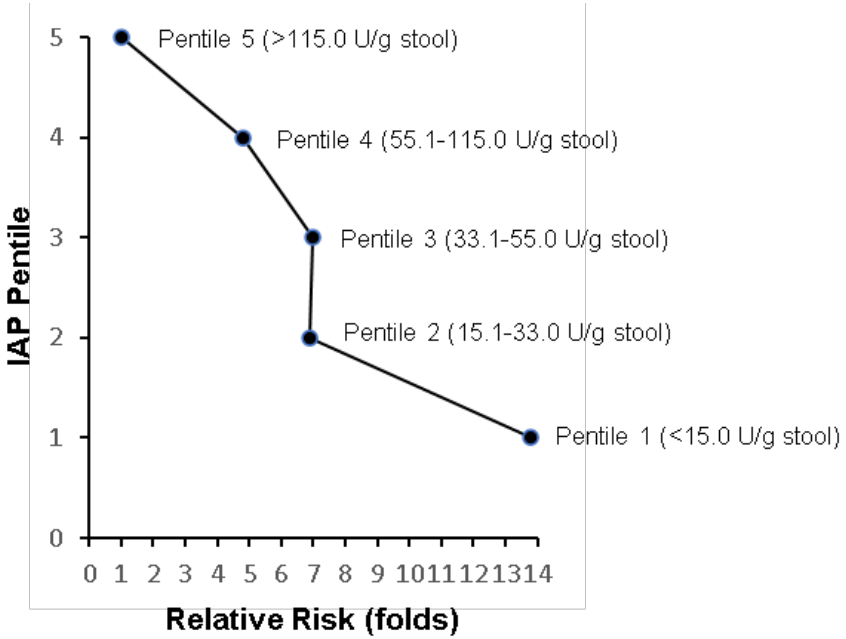
Legend: *All participants, 30 to 60 years old, were stratified into Pentiles based on baseline IAP levels, and then participants with persistent IAP level (during follow-up visit) were identified. Data values are summarized as mean (average) ± SD for each variable. Pentile 5 was the reference Pentile for calculating the statistical significance of difference in the percentage of increase of FPG levels (Student t-test) as well as evaluating age and gender-adjusted relative risk between two Pentiles. *, P<0.05; **, P<0.01; ***, P<0.001.*

The lower the persistent IAPD the higher the risk of T2DM.



Conclusion: IAPD causes diabetes

The lower the persistent IAPD the higher the risk of T2DM.



Conclusion: IAPD causes diabetes

Diabetes Risk Ratio

$$\text{Diabetes Risk Ratio} = \frac{\text{Risk of exposed (IAPD) group}}{\text{Risk of unexposed (no IAPD) group}}$$

$$= \frac{42/100}{3/100} = 14$$

(5-year exposure)

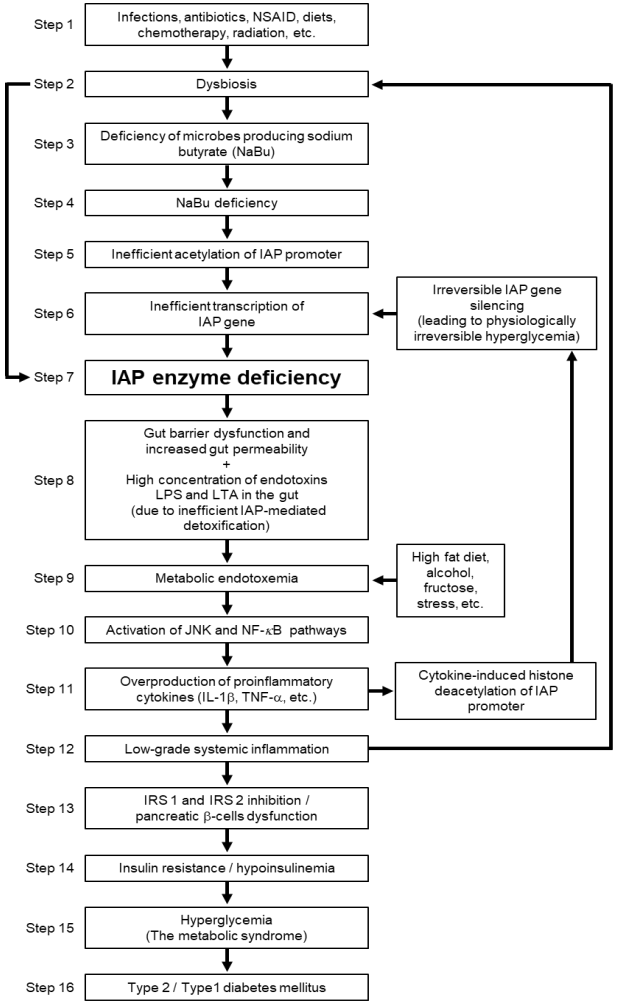
$$\text{Lung Cancer Risk Ratio} = \frac{\text{Risk of smoker group}}{\text{Risk of non-smoker group}}$$

$$= \frac{15/100}{5/100} = 3$$

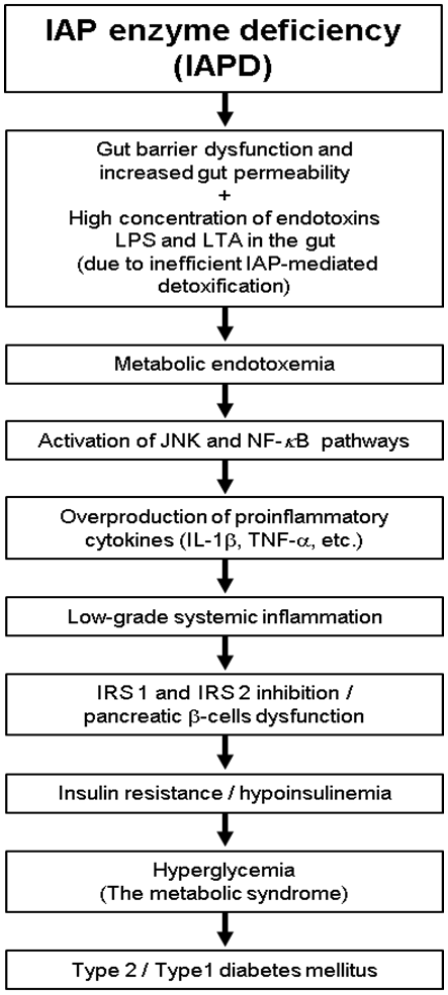
(40-year exposure)

Conclusion: Smoking causes cancer,
so IAPD definitely causes diabetes

Diabetes Pathogenesis Model



IAPD-Mediated Pathogenesis of Diabetes



The Vital Question

Are you going to develop diabetes in the near future?

The Answer

Do the STAP test – know it today!

Availability of STAP test: As soon as the FDA wants!!

(both lab-based and home-based STAP test kits are ready to be available to the public)

Prevention of Diabetes

Current Strategy

1. Screen the population to identify people with IAPD
2. Provide health education (low-fat diet, exercise, drugs, etc.)
3. Avoid unnecessary antibiotics
4. Avoid cold drinks containing fructose
5. May use probiotics (eat yogurt)

Future Strategy

1. Manufacture recombinant IAP
2. Oral supplementation of recombinant IAP
3. Identify IAP activators (compounds that increase IAP production)
4. Clinical trials with IAP activators (such as Turmeric)
5. Use of IAP activators as drugs after successful clinical trials

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Conflict of Interest

Dr. MS Malo is the Chief Scientist
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