Ray A. Kroc & Robert L. Kroc

BDC Lectureship 2014
Ray Kroc (Big Mack)

1902-1984

Predominant establisher of the McDonald's Corporation (1961)

Philanthropist:
Research and treatment of alcoholism, diabetes, MS, arthritis.
Established the Ronald McDonald House foundation.
A major donor to the Dartmouth Medical School.
Robert L. Kroc, PhD
1907-2002

- Ph.D. in Zoology and Physiology from the University of Wisconsin in 1933
- A disciple of Frederick L. Hisaw (the discoverer of relaxin)
- Director of Physiology at the Warner-Lambert Research Institute
  - Proloid® and Euthroid® for the treatment of hypothyroidism
  - Simplastin® for measuring blood-clotting time
  - Releasin® brand of relaxin for the treatment of premature labor
- President of the Kroc Foundation 1969-1985
Type 1 or Type 2 Diabetes: Does This Matter in Youth?

Dorothy J. Becker, MBBCh

Professor of Pediatrics
Director, Diabetes Program at Children’s Hospital of Pittsburgh of UPMC
Dorothy J. Becker, MBBCh

Type 1 or 2 Diabetes: Does it matter in Youth?
No relevant financial relationships with any commercial interests.

Our Pittsburgh Data includes very few Hispanics and no American Indians.
Does it Matter?

- **Pathogenesis** --- yes
  
  But both are heterogeneous

- **Treatment** --- not really
  
  Should treat the individual Insulin requiring vs non-insulin requiring.

We should not be lumpers - rather splitters when we understand more
The best diabetes treatment would alter the root cause

- Reduced beta cell mass and function
- Autoimmune beta cell destruction
- Increased load on remaining beta cells > obesity
- Further beta cell loss
- Insufficient insulin
- Hyperglycemia, DIABETES

Laura Alonso
Diabetes Classification

- Type 1 diabetes
  1a ---- autoimmune marked by islet cell antibodies
  1b ------ idiopathic insulin deficiency
- Type 2 diabetes
- MODY  Maturity-onset diabetes of youth
  Molecular causes of diabetes (known genetic mutations )
- Secondary Diabetes --- cystic fibrosis, drug induced
- Gestational Diabetes
Spectrum of Diabetes

Autoantibodies

ICA GAD IA2 INS

GAD Proinsulin IA2

T Cell Responses

Type I Double Diabetes LADA Type II

Obesity

Insulin Secretion
Spectrum of T1D & IDDM

- Variation of Clinical Phenotype
- Variations of Histopathology
- Intervention Trials
- Variations of Responses
Type 1

Autoimmunity

Type 2

“Double” Diabetes?

MODY

Insulin deficiency

Relative insulin deficiency

Insulin deficiency
Islet autoimmunity in phenotypic type 2 diabetes patients
A

1st phase
p<0.001

2nd phase
p=0.008

OBCN

Ab

Ab+

Insulin (pmol/l)

Time (min)

B

1st Phase
p<0.001

2nd Phase
p<0.001

C-Peptide (nmol/l)

1st Phase

2nd Phase

p<0.001

C

p=0.012

p=0.006

p=0.019

Insulin stimulated glucose disposal (μmol/kg/min)

Total

Oxidative

Non-oxidative

D

Glucose Disposition Index (μmol/Kg/min)

Ab+

Ab-

Control

p < 0.001
ATP/ADP

K

Glucose

Glut 2

Glutamate

GDH

α-Ketoglutarate

Glucokinase

Resting

metabolism

ATP/ADP

SUR1 (Kir 6.2)

K

ATP

K

ATP

SUR1 (Kir 6.2)

Leucine ✔
GTP✔; ADP ✔

Insulin exocytosis

Ca++

Calcium influx

PANCREATIC β CELL

Ca++

Insulin exocytosis

Glucose

Ketoglutarate
NATURAL HISTORY OF “PRE”-TYPE 1 DIABETES

PUTATIVE ENVIRONMENTAL TRIGGER

CELLULAR (T CELL) AUTOIMMUNITY

HUMORAL AUTOANTIBODIES
(ICA, IAA, Anti-GAD65, IA2Ab, etc.)

LOSS OF FIRST PHASE INSULIN RESPONSE
(IVGTT)

GLUCOSE INTOLERANCE
(OGTT)

“PRE”-DIABETES

CLINICAL ONSET

DIABETES

GENETIC PREDISPOSITION

INSULITIS BETA CELL INJURY

BETA CELL MASS

TIME

BETA CELL MASS

TIME
PATHOGENESIS OF TYPE 1 DIABETES

Insulin secretory capacity, %

Environmental factors

Clinical presentation

Age

Environmental factors

Beta-cell autoimmunity

Genetic susceptibility

I.

II.

III.
(Precipitating Event)

Environment

Virus  Diet  Infection  Chemical

Transplacental Exposures

Genetic Predisposition

‘Blueprint’

Microbiome development

Gene expression

Gut dysbiosis that leads to inflammation

Changes in microbiome/metagenomics

Normal insulin release; glucose normal

Progressive loss insulin release

Glucose normal

Changes in metabolic profile and glucose regulation

Overt diabetes

C-peptide present

Residual/No C-peptide

Beta cell mass

Age (years)

BIRTH
PATHOGENESIS OF TYPE 1 DIABETES

I. Genetic susceptibility

II. Trigger
   - 2 months - >20 years
   - IAA, ICA, GADA, ZnT8A, IA-2A, FPIR, IGT

III. Beta-cell autoimmunity

IV. Driving antigen

Insulin secretory capacity, %
Healthy
Clinical disease

Clinical diabetes

Age
0
10-15
2 months - >20 years
100
Cumulative risk of developing clinical Type 1 diabetes in relatives of IDDM probands using Ab markers alone (IAA, GAD65, IA-2, ICA)

Pietropaolo, M et al. Diabetologia 2002; 45: 66-76
Progression to Diabetes From the Time of Seroconversion in Children With Multiple Islet Autoantibodies

Ziegler et al. JAMA 2013; 310: 2473-9
Oral Insulin Did Not Delay Development of T1D

Proportion Free of Diabetes

P- Value = 0.176
(Log Rank Test)

Number at Risk

<table>
<thead>
<tr>
<th>Years Followed</th>
<th>Oral Insulin</th>
<th>Oral Placebo</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>186</td>
<td>186</td>
</tr>
<tr>
<td>1</td>
<td>174</td>
<td>170</td>
</tr>
<tr>
<td>2</td>
<td>146</td>
<td>137</td>
</tr>
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<td>3</td>
<td>110</td>
<td>102</td>
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<td>4</td>
<td>85</td>
<td>71</td>
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<td>5</td>
<td>40</td>
<td>37</td>
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<td>6</td>
<td>23</td>
<td>12</td>
</tr>
<tr>
<td>7</td>
<td>23</td>
<td>12</td>
</tr>
</tbody>
</table>

Delay in T1D was Most Evident in Subjects with Baseline IAA ≥ 300 – Up to 10 years

N=63 (Ins.) and 69 (Plac.)

Log-rank P=0.01
Peto Pr. P=0.01
Hazard Ratio: 0.41 (0.21, 0.80)

Projected 10 year delay

Years Followed

Proportion Free of Diabetes

Oral Insulin
Placebo
Treated
Control

Skyler et al. *Ann NY Acad Sci* 2009; 1150:190-196
Genetics

- Immune System
- Beta Cell
- Insulin sensitivity ---hepatic
  ----peripheral
- Gut ---microbiome
Environment

- Gut
- Obesity
- Antibiotics
- Immunizations
- Toxins
MALT

PLN

OBESITY &
INSULIN RESISTANCE

Apoptosis

Microbiome

Vitamin D ?

Intact Protein

GALT

VITAMIN D ?

Virus

Gluten

Zonulin

Metabolic

HLA

APC, Th1, T regs
Increased intestinal permeability has been reported
- in humans with TIDM
- in experimental pre-diabetic animals (BB rat) & humans

Delayed &/or compromised barrier function in genetically-predisposed individuals may allow passage of antigenic triggers affecting the gut immune system.

Described in other GI autoimmune conditions
- Celiac disease
- Crohn’s disease
# GUT MUCOSAL BARRIER

## Mucosal integrity

<table>
<thead>
<tr>
<th>Rodents</th>
<th>Humans</th>
</tr>
</thead>
<tbody>
<tr>
<td>↑Mucosal permeability</td>
<td>+</td>
</tr>
<tr>
<td>↑Zonulin</td>
<td>?</td>
</tr>
<tr>
<td>Abnormal histology</td>
<td>+</td>
</tr>
<tr>
<td>↓Disaccharidases</td>
<td>+</td>
</tr>
<tr>
<td>Th1 Cytokines</td>
<td>+</td>
</tr>
<tr>
<td>GALT reactivity to food proteins</td>
<td>+</td>
</tr>
</tbody>
</table>
IgA CLASS ANTIBODIES TO COW’S MILK IN TRIGR
THE APPEARANCE OF AT LEAST ONE AUTO-ANTIBODY IN TRIGR

Cumulative Survival Without At Least One Antibody, %

P-Value = 0.366 (Log Rank Test)

Number at Risk:
1078 920 809 748 686 637 529 353 207 82
1073 935 824 761 693 655 555 366 216 85

Casein Hydrolysate
Control Formula

Age (yrs):
0 1 2 3 4 5 6 7 8 9 10

STRATA: --- Casein Hydrolysate --- Control Formula
Lesson II from the NOD mice: The casein hydrolysate does not prevent insulitis

Karges et al. *Diabetes* 1997;46:557-64
Prevalence of overweight by period

- 1980's: 12.6%
- 1990's: 36.8%
- 2000's: 41.1%

p=0.001

Libman 2003
Adipokines and Antigen spreading: Pilot

Baldauff N and Libman I
Autoimmunity

Environment

β-cell damage

Insulin resistance

Clinical Diabetes

Insulin Resistance

Autoimmunity

β-cell damage

Clinical Diabetes


Wilkin et al. *Diabetologia,* 2001
A Model of autoimmune Progression:

- Risk DQ, IDD genes transient T cell resp. (I)
- Several T cell Resp. (II)
- Multiple T cell Resp. ? peri-insulitis (III)
- Progressive pre-IDD plus rat ICA: human ICA GAD Ab IA2 Ab IDDM (IV)

Checkpoints before final β-Cell demise

Approximate % of FDR's

100
30
10
5
0
Recruited new onset subjects
357

≥3 auto-antibody results
285

At least one positive antibody
247 (87%)

Younger had insufficient sample
Percent overweight/obese (>85%ile) by age group

- **Age 0-4**
  - Other: 25.7%
  - African American: 2.9%

- **Age 5-9**
  - Other: 38.7%
  - African American: 3.2%

- **Age 10-14**
  - Other: 29.9%
  - African American: 3.1%

- **Age 15-18**
  - Other: 27.3%
  - African American: 4.5%

Legend:
- Other
- African American
Percent of each antibody group among each age group
No difference in number of antibodies between high and normal BMI

![Bar chart showing percentage of BMI percentile by number of positive antibodies]

- 0-<85 BMI %ile Class
- >=85 BMI %ile Class

- 1: 20.5%
- 2: 33.3%
- 3: 37.2%
- 4: 9.0%
- 1: 15.4%
- 2: 41.8%
- 3: 31.9%
- 4: 11.0%
No difference in number of antibodies by waist percentile
Distribution of antibody positivity in the subgroup with c-peptide < 0.5 ng/ml according to waist circumference (n=24)
The Insulitis Lesion of T1D
T Cell Reactivities in T1D
**Fig. 2.** T-cell responses to islet proteins, using cellular immunoblotting, of children classified with type 1 diabetes, n = 37; type 2 diabetes, n = 19; and indeterminant diabetes, n = 16. *Shaded symbols* indicate individuals positive for at least one autoantibody.
# T cell results clearly discriminate diabetics from controls

<table>
<thead>
<tr>
<th></th>
<th>Diabetes patients (n=261)</th>
<th>Controls (n=45)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years) mean/SD</td>
<td>9.7 (±4.0)</td>
<td>10.6 (±5.1)</td>
<td>0.34</td>
</tr>
<tr>
<td>Race (%white)</td>
<td>92</td>
<td>95</td>
<td>0.09</td>
</tr>
<tr>
<td>Gender (%male)</td>
<td>60</td>
<td>53</td>
<td>0.14</td>
</tr>
<tr>
<td>BMlz Median [IQR]</td>
<td>0.83 [0.26-1.5]</td>
<td>0.86 [0.23-1.66]</td>
<td>0.77</td>
</tr>
<tr>
<td>Waist circumference (cm) Median [IQR]</td>
<td>65 [57-73]</td>
<td>65 [56-77]</td>
<td>0.62</td>
</tr>
<tr>
<td># of positive analytes Median [IQR]</td>
<td>10 [9-10]</td>
<td>0 [0-0]</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>HLA DQ2 and/or DQ8 (%)</td>
<td>81</td>
<td>0</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>
### BMIz at 3 months is higher in the Ab-/T+ subjects

<table>
<thead>
<tr>
<th>Group</th>
<th>Ab+/T-</th>
<th>Ab+/T+</th>
<th>Ab-/T+</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>N</td>
<td>20</td>
<td>213</td>
<td>28</td>
<td></td>
</tr>
<tr>
<td>C-peptide (ng/mL) Median (IQR)</td>
<td>0.5 (0.25-0.7)</td>
<td>0.6 (0.25-0.9)</td>
<td>0.8 (0.25-1.8)</td>
<td>0.08</td>
</tr>
<tr>
<td>Δ C-peptide (ng/mL) Median (IQR)</td>
<td>1 (0.7-1.5)</td>
<td>0.9 (0.2-1.9)</td>
<td>0.87 (-0.1-2.9)</td>
<td>0.8</td>
</tr>
<tr>
<td>BMIz at baseline Mean (SD)</td>
<td>-0.07 (1.1)</td>
<td>0.05 (1.5)</td>
<td>0.8 (1.2)*</td>
<td>0.06</td>
</tr>
<tr>
<td>BMIz at 3 mths Mean (SD)</td>
<td><strong>0.6 (1.1)</strong></td>
<td><strong>0.8 (0.9)</strong></td>
<td><strong>1.4 (0.8)</strong></td>
<td><strong>0.008</strong>*</td>
</tr>
<tr>
<td>Age yrs Mean (SD)</td>
<td>10.3 (3.7)</td>
<td>9.5 (3.9)</td>
<td>11.4 (3.8)</td>
<td>0.5</td>
</tr>
<tr>
<td>Onset A1c% Mean (SD)</td>
<td>11.9 (2.5)</td>
<td>11.8 (2.4)</td>
<td>12.4 (2.1)</td>
<td>0.5</td>
</tr>
</tbody>
</table>
Figure 1: T cell SI in response to each analyte by BMI percentile group
T-cell response is amplified in highest waist percentile for many autoantigens.
Follow-up of T-cell negative
Percent Gaining Ab from baseline to 2 years

<table>
<thead>
<tr>
<th>Category</th>
<th>Percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td>Baseline AA+</td>
<td>2%</td>
</tr>
<tr>
<td>Baseline AA-</td>
<td>10%</td>
</tr>
<tr>
<td>Baseline AA-</td>
<td>46%</td>
</tr>
<tr>
<td>No AA change, all subjects</td>
<td>74%</td>
</tr>
</tbody>
</table>

Purple = Standard AA
Blue = Rat- ICA
Conclusions

- All but one subject had evidence of autoimmunity.

- T-cell autoreactivity to majority of antigens was amplified in those with greatest insulin resistance (waist percentile).

- T-cell autoreactivity in the highest BMI group was amplified in response to neuronal antigens only.
Conclusions

- There was no evidence of a relationship between BMI and T-cell antigen spreading (# of T-cell responses).
- BMI was significantly higher in those without autoantibodies but with abnormal T-cell responses.
- C-peptide was not significantly associated with T-cell or autoantibody status.
The observation that BMIz is highest in T+ autoantibody negative subjects, supports the concept that obesity is associated with diabetes related autoimmunity and may accelerate both onset of clinical diabetes and damage of beta cells prior to the development of conventional autoantibodies.

Could both accelerator hypotheses be correct?
**Accelerator Hypothesis**

**Becker/Libman**

1. Autoimmunity
2. Environment
3. Genes
4. β-cell damage
5. Insulin
6. Insulin Resistance
7. Clinical Diabetes

**Wilkin**

1. Insulin Resistance
2. Autoimmunity
3. β-cell damage
4. Clinical Diabetes
Acknowledgements

Fellows  Melissa Buryk
         Natalie Baldauff

Ingrid Libman
Michael Dosch
Massimo Pietropaulo

Diabetes Research Nurses and Technicians
HR estimates for effect of BMI on all-cause mortality.

Logue J et al. Dia Care 2013;36:887-893
Effective Insulin Dose

NOD MOUSE
- 1mg --- protective (40mg/kg)
- 0.3 mg --- ineffective
- 2mg --- accelerate (80mg/kg)

BB RAT
- 2mg --- ineffective (8mg/kg)

HUMAN
- 7.5mg --- ineffective (0.15mg/kg adult)
- ?effective (0.4mg/kg child)
Older subjects have higher levels of C-peptide at 3 months.
Similar number of positive antibodies irrespective of BMI class

![Graph showing percentage of positive antibodies across BMI classes](Image)
Development of at least one autoantibody by age

P = 0.03

Cumulative survival without one autoantibody

Age years

0 1 2 3 4 5 6 7 8

INTERVENTION (HF) 91 86 82 77 71 38
CONTROLS (CF) 101 97 94 84 72 34
BMIz is higher in T-cell positive/antibody negative than antibody positive subjects.
Figure 3: T cell SI comparing waist <25th percentile and >85th percentile
Change in Ab status from baseline to 2 years

- Any AA: Baseline 89%, 2 year F/U 84%
- GAD: Baseline 55%, 2 year F/U 43%
- ICA: Baseline 80%, 2 year F/U 70%
- IA2: Baseline 71%, 2 year F/U 66%

Lost any AA: 26%
Lost all AA: 5%
Percent of antibody positive subjects decreases over time

- IA2
- Gad
- ICAh
- ICAr

P-trend = 0.06

P-trend = 0.007
Percent of BMI %ile ≥85 by age and race:

- **Age 0-4**
  - White: 25.7%
  - Black: 2.9%
  - Other: 3.2%
  - Total: 31.8%

- **Age 5-9**
  - White: 38.7%
  - Black: 3.1%
  - Other: 1.1%
  - Total: 43.0%

- **Age 10-14**
  - White: 29.9%
  - Black: 4.5%
  - Other: 3.1%
  - Total: 37.5%

- **Age 15-18**
  - White: 27.3%
  - Black: 9.1%
  - Other: 4.5%
  - Total: 41.9%

*Libman*
### T cell results clearly discriminate diabetics from controls

<table>
<thead>
<tr>
<th>Analyte</th>
<th>Usage</th>
<th>New onset diabetes (n=261) Median SI [IQR]</th>
<th>FDR controls (n=45) Median SI [IQR]</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gad</td>
<td>Test analyte</td>
<td>2.0 [1.7-2.3]</td>
<td>1.1 [1-1.2]</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Gad55</td>
<td>Test analyte</td>
<td>2.0 [1.7-2.3]</td>
<td>1.1 [1-1.2]</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>PI</td>
<td>Test analyte</td>
<td>2.1 [1.8-2.4]</td>
<td>1.1 [1-1.3]</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Tep69</td>
<td>Test analyte</td>
<td>2.1 [1.8-2.4]</td>
<td>1.1 [1-1.2]</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>MBP</td>
<td>Test analyte</td>
<td>1.8 [1.6-2.0]</td>
<td>1.1 [1-1.2]</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>EX2</td>
<td>Test analyte</td>
<td>1.8 [1.6-2.0]</td>
<td>1.1 [1-1.2]</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>GFAP</td>
<td>Test analyte</td>
<td>2.0 [1.7-2.2]</td>
<td>1.1 [1-1.2]</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>S100</td>
<td>Test analyte</td>
<td>1.9 [1.6-2.2]</td>
<td>1.1 [1-1.2]</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>ABBOS</td>
<td>Test analyte</td>
<td>2.1 [1.9-2.4]</td>
<td>1.1 [1-1.3]</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>BSA</td>
<td>Test analyte</td>
<td>2.2 [1.9-2.5]</td>
<td>1.1 [1-1.1]</td>
<td>&lt;0.001</td>
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<tr>
<td>PHA</td>
<td>Positive control, proliferation competence</td>
<td>23 [19-26]</td>
<td>21 [17-26]</td>
<td>0.1</td>
</tr>
<tr>
<td>TT</td>
<td>Positive control, post-vaccination response competence</td>
<td>10 [8-11]</td>
<td>9 [7-10.5]</td>
<td>0.07</td>
</tr>
<tr>
<td>OVA</td>
<td>Negative control</td>
<td>1.0 [1-1.1]</td>
<td>1.0 [1-1.1]</td>
<td>0.25</td>
</tr>
<tr>
<td>Actin</td>
<td>Negative control</td>
<td>1.1 [1-1.1]</td>
<td>1.0 [1-1.1]</td>
<td>0.1</td>
</tr>
</tbody>
</table>
BMI percentiles at 3 months

BMI%ile at 3 months

Percentage

- Other
- African American
- Caucasian

0  5  10  15  20  25  30  35  40

BMI%ile at 3 months
Age distribution by race

- Caucasian n=231
- African American n=13
- Other n=3

Age (years)

Frequency
No association of waist circumference percentile and age at onset
<table>
<thead>
<tr>
<th>Group</th>
<th>Ab+/T+</th>
<th>Ab-/T+</th>
<th>Ab+/T-</th>
<th>P value</th>
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<tr>
<td>N</td>
<td>173</td>
<td>27</td>
<td>15</td>
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<td>C-peptide (ng/mL) Median [IQR]</td>
<td>0.6 [0.25-0.9]</td>
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<td>0.08</td>
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<td>Δ C-peptide (ng/mL) Median [IQR]</td>
<td>0.9 [0.2-1.9]</td>
<td>0.87 [-0.1-2.9]</td>
<td>1 [0.7-1.5]</td>
<td>0.8</td>
</tr>
<tr>
<td>BMIz at baseline Mean ± SD</td>
<td>0.05 ±1.5</td>
<td>0.8 ± 1.2*</td>
<td>-0.07 ±1.1</td>
<td>0.046*</td>
</tr>
<tr>
<td>BMIz at 3 mths Mean ± SD</td>
<td>0.8 ± 0.9</td>
<td>1.4 ± 0.8</td>
<td>0.6 ±1.1</td>
<td>0.008*</td>
</tr>
<tr>
<td>Age yrs Mean ± SD</td>
<td>9.5 ± 3.9</td>
<td>11.4 ± 3.8</td>
<td>10.3 ± 3.7</td>
<td>0.5</td>
</tr>
<tr>
<td>Onset HbA1c% Mean ± SD</td>
<td>11.8 ± 2.4</td>
<td>12.4 ± 2.1</td>
<td>11.9 ± 2.5</td>
<td>0.5</td>
</tr>
</tbody>
</table>