The involvement of mitochondria in chronic low-grade inflammation associated with maltreatment experiences during childhood

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Childhood maltreatment (CM) is a worldwide phenomenon present in all classes of low- and high-income countries

Child maltreatment includes all forms of physical and emotional ill-treatment, sexual abuse, neglect, and exploitation that results in actual or potential harm to the child’s health, development or dignity (WHO)

-> five subtypes

- International prevalence estimates of childhood maltreatment
  - Physical abuse: 25% of all adults
  - Sexual abuse: 20% women and 8% of all men
  - Emotional & physical neglect most prevalent forms of CM

- Chronic (stress) condition
  - Re/Poly-victimization

- *Childhood Trauma Questionnaire (CTQ)*

**Childhood Trauma Questionnaire (CTQ)**

The self-report includes a 28-item test that measures the 5 subtypes of childhood maltreatment (age < 18 years) with a 5-point Likert-scale.

<table>
<thead>
<tr>
<th>When I was growing up...</th>
<th>Never True</th>
<th>Rarely True</th>
<th>Sometimes True</th>
<th>Often True</th>
<th>Very Often True</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. I didn’t have enough to eat.</td>
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<tr>
<td>2. I knew that there was someone to take care of me and protect me.</td>
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<td>3. People in my family called me things like “stupid,” “lazy,” or “ugly”.</td>
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<td>4. My parents were too drunk or high to take care of the family.</td>
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<td>5. There was someone in my family who helped me feel that I was important or special.</td>
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<tr>
<td>6. I had to wear dirty clothes.</td>
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<td>7. I felt loved.</td>
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<tr>
<td>8. I thought that my parents wished I had never been born</td>
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<tr>
<td>9. I got hit so hard by someone in my family that I had to see a doctor or go to the hospital.</td>
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<tr>
<td>10. There was nothing I wanted to change about my family</td>
<td></td>
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<tr>
<td>11. People in my family hit me so hard that it left me with bruises or marks.</td>
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<tr>
<td>12. I was punished with a belt, a board, a cord, or some other hard object.</td>
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</tr>
</tbody>
</table>
Aversive childhood experiences have long-life negative consequences on the risk for somatic as well as mental health conditions.

Adverse experiences during childhood

Social environment: unpredictable and threatening

Adaptation of nervous, endocrine, and immune systems

Cumulative stress exposure, life events, and environment

Detrimental effects on health in the long run

Increased stress load in every day life

Psychological level

Physiological level

Risky health behavior

PTSD, Depression, Anxiety Disorder, Substance abuse

Arthritis, cancer, cardiovascular diseases, diabetes

Chronic and traumatic stress, including adverse childhood experiences (ACE), increase the risk for various physical diseases and diminishes life quality.

Seng et al. (2006), *Journal of Traumatic Stress*
Felitti et al. (1998), *American Journal of Preventive Medicine*
ACEs show a dose-response effect on the risk for mental pathology, especially major depression and anxiety disorder.

Child abuse and neglect increase the risk for:

- Reduced health-related life quality
- Unsecure or desorganized psychosocial attachment
- Depression
- Anxiety disorders
- Alcohol abuse / dependency
- Drug abuse / dependency
- Eating disorders
- Posttraumatic stress disorder
- Dissociative disorder
- Personality disorder
- Suicide attempts

Chapman et al. (2004), *Journal of Affective Disorders*
Li et al. (2016), *Psychological Medicine*
A history of childhood maltreatment is associated with a higher risk for immunological impairments.

- Chronic low-grade inflammation
- Impaired wound repair
- Impaired cell-mediated immunity
- Weakened immune response
- Altered composition of peripheral immune cells
- Auto-immunity

Coelho et al. 2014; O’Connor et al. 2014; Godbout & Glaser 2006; Danese & McEwen 2011; Wegman et al. 2009; Danese et al. 2007

Besides they bioenergetic functioning, mitochondria play a key role in immunity and inflammation. So far, they role in CM has not been investigated on a functional level.

Are mitochondrial alterations involved in the establishment of the pro-inflammatory phenotype with child maltreatment experiences?
“My Childhood – Your Childhood”: The influence of childhood experiences on mothers and their infants in a transgenerational context.

**Maternity hospital Ulm**
- Birth
  - $t_0$: day 1-3 post partum
  - Informed consent
  - Screening interview
  - Study inclusion
  - Biosampling: (Umbilical chord) blood, hair, buccal cells: mother and child

**Clinical & Biological Psychology**
- $t_1$: 3 months post partum
  - Psychodiagnostic interview:
    - Child maltreatment
    - Traumatic life events
    - Psychopathology
    - Attachment representation
    - Social support
    - Current stress
    - Sociodemographic information
  - Biosampling:
    Blood (only mother)
    Buccal cells

**Child & Adolescent Psychiatry**
- $t_2$: 12 months post partum
  - Psychological diagnostics of the child:
    - Developmental status
    - Attachment representation
  - Psychological diagnostics of the mother:
    - Current stress and parental strain
    - Social support and usage of support systems
  - Biosampling:
    Buccal cells, saliva, hair

**“My Childhood – Your Childhood”**
- The influence of childhood experiences on mothers and their infants in a transgenerational context.
Demographical and psychological data: “My Childhood – Your Childhood”

<table>
<thead>
<tr>
<th>Demographics</th>
<th>Total (N = 30)</th>
<th>CTQ classification</th>
<th>p^e</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>None (N = 8)</td>
<td>Low/moderate (N = 11)</td>
</tr>
<tr>
<td>Age (years)</td>
<td>31.6 ± 6.0</td>
<td>32.5 ± 4.5</td>
<td>33.6 ± 6.7</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>25.3 ± 6.4</td>
<td>26.6 ± 7.7</td>
<td>26.3 ± 8.0</td>
</tr>
<tr>
<td>Smoking status (yes, n (%))</td>
<td>8 (26.7%)</td>
<td>1 (12.5%)</td>
<td>3 (27.3%)</td>
</tr>
<tr>
<td>Alcohol consumption (yes, n (%))</td>
<td>10 (33.3%)</td>
<td>3 (37.5%)</td>
<td>3 (27.3%)</td>
</tr>
<tr>
<td>Physical activity (yes, n (%))</td>
<td>8 (26.7%)</td>
<td>3 (37.5%)</td>
<td>3 (27.3%)</td>
</tr>
<tr>
<td>Ethnicity (Caucasian, n (%))</td>
<td>29 (96.8%)</td>
<td>8 (100.0%)</td>
<td>11 (100.0%)</td>
</tr>
</tbody>
</table>

**Adversity and psychiatric symptom load**

<table>
<thead>
<tr>
<th>CTQ sum score</th>
<th>42.8 ± 14.2</th>
<th>28.3 ± 1.7</th>
<th>37.7 ± 4.5^d</th>
<th>58.6 ± 9.7^de</th>
<th>&lt;0.001</th>
</tr>
</thead>
<tbody>
<tr>
<td>Emotional abuse sum score</td>
<td>9.8 ± 5.3</td>
<td>5.6 ± 0.9</td>
<td>7.6 ± 2.1</td>
<td>15.0 ± 5.1^de</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Physical abuse sum score</td>
<td>7.1 ± 3.8</td>
<td>5.3 ± 0.5</td>
<td>5.7 ± 1.6</td>
<td>9.9 ± 5.0^de</td>
<td>0.005</td>
</tr>
<tr>
<td>Sexual abuse sum score</td>
<td>6.7 ± 4.1</td>
<td>5.0 ± 0</td>
<td>5.8 ± 2.1</td>
<td>8.8 ± 6.1</td>
<td>0.09</td>
</tr>
<tr>
<td>Emotional neglect sum score</td>
<td>12.3 ± 4.8</td>
<td>7.4 ± 1.7</td>
<td>12.6 ± 2.6^d</td>
<td>15.6 ± 5.1^d</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Physical neglect sum score</td>
<td>6.9 ± 2.9</td>
<td>5.0 ± 0</td>
<td>6.0 ± 1.1</td>
<td>9.2 ± 3.8^d</td>
<td>0.002</td>
</tr>
<tr>
<td>PSS sum score</td>
<td>4.1 ± 2.8</td>
<td>3.5 ± 2.4</td>
<td>3.0 ± 2.4</td>
<td>6.2 ± 2.4</td>
<td>0.06</td>
</tr>
<tr>
<td>HADS depression sum score</td>
<td>3.8 ± 3.2</td>
<td>2.8 ± 2.1</td>
<td>3.6 ± 2.5</td>
<td>4.9 ± 4.3</td>
<td>0.34</td>
</tr>
<tr>
<td>HADS anxiety sum score^d</td>
<td>6.3 ± 3.5</td>
<td>5.1 ± 2.6</td>
<td>4.5 ± 2.8</td>
<td>8.9 ± 3.2</td>
<td>0.004</td>
</tr>
</tbody>
</table>
Short workflow for cryopreservation of PBMC and sample processing for high-resolution respirometry using O2K-Oxygraphs.

Cryopreservation in short

1. Wash (3x) in PBS (1200 rpm, 10 min, RT)
2. max. 7.5 x 10^6 cells/ ml freezing medium
FCS: DMSO (10:1)

1 x 10^6 living cells

Mito-density
CSA
Overview – Biological analyses

Mitochondrial respiration (bioenergetics) & citrate synthase activity (marker for mitochondrial density per cell)

Spontaneous secretion of pro-inflammatory cytokines ex vivo

37°C, 5% CO₂, 24 h

CRP level as serum marker for systemic inflammation

Level of anti-inflammatory metabolites (lysoPC) and endogenous markers of oxidative stress (Arg:Cit ratio, antioxidants Acetylcholine and L-Carnitine)
Inflammatory markers are positively associated with maltreatment load, while anti-inflammatory markers show a negative association with CM.
Mitochondrial activity of PBMC increases with higher severity of CM experiences in a dose-dependent manner.

Basal ("routine") respiration of intact PBMC

Oxygen consumption related to ATP-turnover

Oxygen consumption attributable to proton leak

Citrate synthase activity (mitochondrial density)
More severe CM experiences are dose-dependently associated with higher levels of biovariables related to oxidative stress.

Non-OXPHOS oxygen consumption of intact PBMC

Arginine:citrulline ratio

Serum L-carnitine level

Serum acetylcarnitine level

$\beta = .37$

$p = .03$

$\beta = .29$

$p = .04$

$\beta = -.38$

$p = .04$

$\beta = -.41$

$p = .07$
Mitochondrial basal respiration, ATP-turnover related respiration and the residual oxygen consumption significantly correlate with the release of pro-inflammatory cytokines in vitro

<table>
<thead>
<tr>
<th>Basal (“routine”) respiration</th>
<th>IL1β</th>
<th>IL6</th>
<th>TNFα</th>
</tr>
</thead>
<tbody>
<tr>
<td>Routine Control Ratio + Smoking Status</td>
<td>![triple dot]</td>
<td>![triple dot]</td>
<td>![triple dot]</td>
</tr>
<tr>
<td>Smoking Status</td>
<td>![dot]</td>
<td>![dot]</td>
<td>![dot]</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>ATP-turnover related respiration</th>
<th>IL1β</th>
<th>IL6</th>
<th>TNFα</th>
</tr>
</thead>
<tbody>
<tr>
<td>NetRoutine Ratio + Smoking Status</td>
<td>![dot]</td>
<td>![dot]</td>
<td>![dot]</td>
</tr>
<tr>
<td>Smoking Status</td>
<td>![dot]</td>
<td>![dot]</td>
<td>![dot]</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>ROS-related marker</th>
<th>IL1β</th>
<th>IL6</th>
<th>TNFα</th>
</tr>
</thead>
<tbody>
<tr>
<td>Residual Control Ratio + Smoking Status</td>
<td>![green dot]</td>
<td>![blue dot]</td>
<td>![green dot]</td>
</tr>
<tr>
<td>Smoking Status</td>
<td>![dot]</td>
<td>![dot]</td>
<td>![dot]</td>
</tr>
</tbody>
</table>

**p < .01**, *p < .05*, (*) p < .10, **p > .10**
Summary

PBMC mitochondrial activity

ROS

IL-6
IL-1β
TNF-α

Inflammation

CRP

Oxidative stress

Lysophosphatidylcholines

Acetyl carnitine

L-Carnitine

Citrulline + NO synthase

Arginine
Thank you for your attention!

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